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Report of the

ICES-IOC Working Group on Harmful Algal Bloom Dynamics

Bermuda
7–10 March 2002

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 WELCOME AND OPENING OF THE MEETING

The meeting of the ICES-IOC Working Group of Harmful Algal Bloom Dynamics was organised and chaired by Allan Cembella (Canada), in place of Kaisa Kononen who was unable to attend the meeting. She has taken a position within the Finnish Academy of Sciences and is unable to continue as Chair of the Working Group. The Working Group meeting was held at the Bermuda Biological Station for Research (BBSR) from 7–10 March 2002. Thirteen scientists from 11 countries participated. The list of participants is presented in Annex 1. The meeting agenda is presented in Annex 2.

Allan Cembella opened the meeting at 09:00 on 7 March 2002 and the participants were introduced with respect to their names, institutional and national affiliations, and field of expertise.

2 TERMS OF REFERENCE

The Terms of Reference for the WGHABD 2002 were approved by the Oceanography Committee of ICES through Resolution Cm2001/2C03 at the 89th Statutory Meeting, Oslo, Norway.

The **ICES-IOC Working Group on Harmful Algal Bloom Dynamics** [WGHABD] (Chair: Dr K. Kononen, Finland) will meet in Bermuda, from 7–10 March 2002 to:

a) collate and assess national reports, update the decadal mapping of harmful algal events for the IOC-ICES harmful event database (HAE-DAT) on a regional, temporal and species basis, and specifically examine the 15-year time series for possible temporal trends or regional distribution patterns;
b) review GEOHAB implementation in the ICES area;
c) review existing data on the identification, distribution and toxicological significance of new and emerging phycotoxins and causative organisms, in terms of human health significance, HAB population dynamics, and effects on marine food webs;
d) continue examining the ways of analysing historical data;
e) review progress in the organization of a workshop on real-time observation systems in coastal ecosystems for studies of harmful algal blooms;
f) evaluate progress in the application of molecular probe technologies for a) taxonomic and genetic studies, b) the detection and enumeration of HAB species, and c) the investigation of their physical condition;
g) report and discuss new findings;
h) prepare a summary report listing relevant marine bio-ecological variables and indicators suitable for operational use.

3 EXECUTIVE SUMMARY AND CONCLUSIONS

Term of Reference a: Collate and assess national reports, update the decadal mapping of harmful algal events for the ICES-IOC Harmful Algal Event Database (HAE-DAT) on a regional, temporal and species basis, and specifically examine the 15-year time series for possible temporal trends or regional distribution patterns.

The national representatives of each ICES country presented the national reports.

The format of the decadal maps was discussed at length and a number of recommendations for improvements were made. The proposed addition of yearly maps to allow the examination of annual trends was also discussed.

Results from the HAEDAT database were presented. The current forms require a lot of proofing by the database administrator prior to entry into the database. A number of issues concerning the quality of data entered on the form were highlighted, particularly with respect to reporting protocols that interfered with analysis of the data. Two terms of reference were proposed by the Working Group for further consideration: 1) to review previous forms with a view to improving the quality of the data extracted by the end users, and 2) to investigate the possibility of creating maps from the HAEDAT forms using GIS.

Term of Reference b: Review GEOHAB implementation in the ICES area.

Presentations were made on GEOHAB implementation in the Baltic region, Canada, Europe and China.

Bengt Karlson (Sweden) presented a report of the SSGGIB Study Group on GEOHAB implementation in the Baltic. This Study Group had compiled a proposal for research on HABs in the Baltic Sea. The need for funding to accomplish this was discussed by the WGHABD.

Jennifer Martin (Canada) reported on a preliminary workshop held in Montreal in October 2001 to co-ordinate interest in scientific research in relation to developing a better understanding of HABs by Canadian scientists. This Canadian initiative would cover population dynamics, biological growth/losses, and observation/prediction systems relating to key HAB species in Canadian waters. A further workshop is planned for August 2002 to consolidate efforts into the production of an explicit Canadian GEOHAB research proposal with participation of international partners.

Beatriz Reguera (Spain) reported on the LIFEHAB Workshop on life-history strategies of HAB species held in Calviá (Mallorca, Spain) in October 2001, funded by the Commission of the European Union (CEU). The objectives of the meeting were to summarise current knowledge on the life history of harmful species, to identify the main gaps of knowledge, and to discuss the most appropriate approaches and methods to address the role of life cycles in HAB dynamics. The Workshop is directly relevant to one of the GEOHAB themes on Adaptive Strategies. The proceedings with extended abstracts of key presentations, other contributions and discussion groups at this meeting will be made available to the public on the Internet, and a booklet will also be produced by the CEU.

Don Anderson (USA) provided information on the initiation of GEOHAB-related research in Chinese coastal waters. China has just funded a five-year Chinese Ecology and Oceanography of Harmful Algal Blooms Programme (CEOHAB). This is a national research programme with foreign involvement in an advisory role. As such, this is not seen as a true GEOHAB programme at this stage, but the initiative should be encouraged and supported. China presents a unique opportunity for the study of eutrophication effects on HABs. The WG recommends that the GEOHAB scientific Steering Committee take an active role in formulating and eventually implementing an international programme dealing with high biomass blooms along the Chinese coast.

Term of Reference c: Review existing data on the identification, distribution and toxicological significance of new and emerging phycotoxins and causative organisms, in terms of human health significance, HAB population dynamics, and effects on marine food webs.

Several presentations were given on the topic of new and emerging phycotoxins. Allan Cembella (Canada) and Bernd Luckas (Germany) reviewed the identification, distribution and toxicological significance of new and emerging phycotoxins in the ICES area, focusing on toxins discovered recently in microalgae and shellfish from Europe and North America, particularly on azaspiracids (AZAs), spirolides, novel DSP toxins, yessotoxins (YTXs) and pectenotoxins (PTXs). The history and identification of these emerging toxins by chemical analytical methods was presented and the methods used to identify the causative organisms were described.

Among the emerging natural toxins accumulated in seafood, YTXs and PTXs are often grouped together with toxins responsible for DSP, i.e., okadaic acid (OA) and dinophysistoxins (DTXs). Over the last decade, YTXs and PTXs have caused growing concern among public health authorities as well as in the shellfish industry. These emerging toxins are a controversial topic among scientists and health officials, as "DSP toxin" contamination proves to be more complex than originally believed. YTXs and PTXs are now known to be widely distributed in ICES countries, rather than being restricted to Japan, where they were discovered. Whether this is owing to an actual but natural expansion of the "DSP phenomenon" from bloom advection, to the global transfer of shellfish stock, or to increased statutory monitoring measures and scientific investigations remains to be determined. However, urgent sanitary measures are required to protect consumers' health from the ingestion of YTX- and PTX-contaminated seafood, and to secure the shellfish industry from the significant economic damages due to extensive closures. A reclassification of the polyether (lipophyllic) toxins, so far grouped under the term "diarrhetic toxins" (DSP), and new regulation levels and control methods have been established by the Commission of the European Union. An amendment of the CEU Directive 91/492 concerning "DSP toxins" has just been published in the "Official Journ of the European Communities" as the Commission Decision 2002/226/EC.[1]

Joe Silke (Ireland) reviewed azaspiracid poisoning (AZP) in Ireland. Shellfish harvesting areas were closed again in 2001 due to high levels of AZA in shellfish. The toxicological effects of this toxin have been further investigated and results from these studies need to be considered by public health officials and the seafood industry.

[1] Commission Decision of 15 March 2002 laying down detailed rules for the implementation of Council Directive 91/492/EEC as regards the maximum levels and the methods of analysis of certain marine biotoxins in bivalve molluscs, echinoderms, tunicates and marine gastropods .

Einar Dahl (Norway) reported briefly on YTX contamination of shellfish in Norway. Improved methods for the detection of lipophilic toxins in Norway resulted in an increase in the number of DSP and YTX toxins detected. *Protoceratium reticulatum* (=*Gonyaulax grindleyi*) was identified as the causative organism for YTX contamination of shellfish in Norway in 2001. In related information presented as "New Findings", investigations into fish mortalities resulting from a *Chattonella* bloom in Norway showed no toxicity present. The fish-killing dinoflagellate *Pfiesteria* was also recently isolated from sediment from the Oslofjord and found to be toxic, but the toxicity is not well defined.

In summary, key information concerning new and emerging phycotoxins is still lacking. While methods for the detection of phycotoxins have improved in recent years, the causative organism for certain toxins, such as AZA, has yet to be confirmed. One potential source organism for AZA, the dinoflagellate *Protoperidinium* spp. has been implicated as the causative agent; however, there are methodological weaknesses with the technique used to determine this relationship. *Protoceratium reticulatum* and *Lingulodinium polyedra* have both been identified as YTX-producers, but it is also possible that there are other sources. Future work on emerging toxins should focus on the cause and effect linkages between toxin source organisms and their association with shellfish and finfish toxicity.

Term of Reference d: Continue examining the ways of analysing historical data.

Analysis of historical data from the Bay of Fundy was presented by Jennifer Martin (Canada). Multidimensional scaling (MDS) of data collected since 1991 shows that spatial differences do exist between stations and that there is a seasonal pattern of change in community structure. This analysis will be extended to evaluate annual variability and possible comparisons between regions and data sets. A term of reference has been proposed by the WG to identify time-series data sets available to allow for comparable analysis of historical data.

Term of Reference e: Review progress in the organisation of a workshop on real-time observation systems in coastal ecosystems for studies of harmful algal blooms.

Progress from the organisational meeting held in Villefranche, France in February 2002 was reported by Bengt Karlson (Sweden). The Conveners and Organising Committee members were identified and a provisional agenda of the workshop was considered by the WG. The format of the workshop was presented and the results of the meeting were discussed. The IOC has expressed interest in publishing a book from the proceedings of this meeting, possibly within the IOC Monograph series.

Term of Reference f: Evaluate progress in the application of molecular probe technologies for a) taxonomic and genetic studies, b) the detection and enumeration of HAB species, and c) the investigation of their physiological condition.

Don Anderson (USA) and Allan Cembella (Canada) made presentations to the WG on this topic dealing with the development of probes for phytoplankton species and toxins. The development of taxon-specific probes for key HAB species, using antibodies, nucleotide sequences, and lectins was discussed at length. Results from the implementation of these probes during field trials were presented. Probes are also being developed for use as biochemical indicators for physiological processes in the cell.

A number of toxin-specific probes based upon antibody, receptor-binding and cytotoxicity assays developed for detection of toxins in shellfish and fish tissues, are now being re-formatted for use with plankton extracts. A few of these assay methods have been applied as probes to localise toxins within phytoplankton cells, but there are many technical limitations to this approach.

The technology of molecular probes has advanced considerably in recent years, driven by the need to identify, enumerate, separate or physiologically characterise HAB species. Probes of various types have been developed, and these have been applied in a diverse array of assay types. Some approaches are now being used in routine monitoring or research on specific HAB species, but others remain in the laboratory at the developmental stage. As efforts have been made to apply probes to natural populations, problems have arisen; some have been resolved, but others remain. Despite these occasional setbacks, the technology is powerful and should continue to contribute to rapid progress in studies on HAB taxa and their toxins.

Term of Reference g: Report and discuss new findings.

Three presentations on new findings were made by WG members, including a presentation on the use of acoustic sensors for the discrimination of thin-layer phenomena and zooplankton aggregation given by Percy Donaghay (USA).

Don Anderson (USA) announced several scientific programmes relevant to ECOHAB in the USA with linkages to European initiatives.

Term of Reference h: Prepare a summary report listing relevant marine bio-ecological variables and indicators suitable for operational use.

The WG recognized the importance of defining the relevant bio-ecological variables for studies of HAB dynamics, but background information on this ToR was not provided to the members for consideration in advance of the 2002 meeting. Nevertheless, the WG initiated a preliminary discussion on this topic for future consideration with external expertise. To some extent, this subject was already addressed in invited presentations on bio-optics and remote sensing at the 2001 WGHABD meeting in Dublin (see ICES WG Report on ToR 4 by John J. Cullen (Canada) and Jim Aiken (UK)). Within the WGHABD, a consensus developed that the definition of relevant marine bio-ecological variables and the implementation of their measurement would be fully explored at the planned Workshop on Real-Time Observation Systems in Coastal Ecosystems for Studies of Harmful Algal Blooms in June, 2003 at Villefranche, France, and in the subsequent publication and web-based information. Two members of the WGHABD (Allan Cembella (Canada) and Bengt Karlson (Sweden)) are also members of the Organising Committee for this Workshop and will ensure that advances on this topic are adequately brought to the attention of the WG.

4 NATIONAL REPORTS AND HAEDAT-DATABASE

Term of Reference a: Collate and assess national reports, update the decadal mapping of harmful algal events for the IOC-ICES harmful event database (HAE-DAT) on a regional, temporal and species basis, and specifically examine the 15-year time series for possible temporal trends or regional distribution patterns.

4.1 National Reports

Representatives from Latvia and the Netherlands did not attend but submitted national reports to IOC-IEO SCCHA, Vigo.

4.1.1 Canada

4.1.1.1 West Coast

As usual, the west coast of Canada experienced annual, widespread PSP toxin events, as characterized by high shellfish toxicity. Other HAB events in the past year included a number of salmon mortalities at aquaculture sites. Locations, species implicated and timing of events were as follows: *Chaetoceros concavicornis* levels of 1.7 X 10^5 cells l^{-1} in mid-April from northeast Vancouver Island; *C. convolutus* concentrations of 1.7 X 10^5 cells l^{-1} in southeast Vancouver Island in mid-May; during mid – late August, *Heterosigma akashiwo* concentrations of 1.0 X 10^8 cells l^{-1} in the Clayoquot Sound region of Vancouver Island; *Chrysochromulina* sp. in the northeast Vancouver Island area at concentrations of 2.0 X 10^7 cells l^{-1} causing water discolouration during the first week of September; and mass mortalities of salmon during the first two weeks of September. The species responsible for the latter incident was not determined, although *Chaetoceros tenuissimus* was observed at concentrations of 7.0 X 10^7 cells l^{-1} in the Kyuquat Sound region of Vancouver Island.

4.1.1.2 East Coast

The east coast of Canada is divided into five regions for HAB and toxin monitoring – the St. Lawrence Estuary, Gulf of St. Lawrence, Bay of Fundy, southern Nova Scotia and Newfoundland. In the St. Lawrence Estuary, shellfish harvesting areas were closed as a result of unacceptably high levels of PSP toxicity. An *Alexandrium tamarense* bloom extended from early June through mid August with observed concentrations as high as 1.0 X 10^6 cells l^{-1}. Areas were also closed to harvesting from May through August as a result of unacceptably high levels of DSP toxins in the northeast part of the Gulf of St. Lawrence, but the causative organism was not determined. High levels (340 µg g^{-1}) of domoic acid were measured in scallop gonads and digestive glands during July, August and September.

Malpeque Bay, Prince Edward Island (Gulf of St. Lawrence) had closures as a result of domoic acid levels as high as 27 µg g^{-1} from late October to mid-November. The species of *Pseudo-nitzschia* responsible requires further SEM work for confirmation and identification. Northern Nova Scotia experienced its first closures as a result of domoic acid, which reached a level of 21 µg g^{-1} in December (the causative organism has yet to be determined).

In St. Margaret's Bay, on the southwest coast of Nova Scotia, there were closures due to PSP toxicity levels as high as 684 µg $100g^{-1}$ shellfish in June and July. High levels of PSP toxicity resulted in additional closures at Indian Point in May, and in Shelburne from July to August. The species of *Alexandrium* was not identified.

Areas around Digby on the northwest coast of Nova Scotia were closed from July to October owing to high PSP toxicity. Levels as high as 1185 µg g^{-1} were recorded in blue mussels.

Additional areas within the Bay of Fundy were closed to harvesting owing to the presence of *Alexandrium fundyense* and consequent PSP toxicity (up to 2600 µg $100g^{-1}$ in blue mussels [*Mytilus edulis*] and 1000 µg $100g^{-1}$ in soft-shell clams [*Mya arenaria*] during May–July.

Unacceptably high levels of DSP toxins in Mahone Bay, NS produced by an unidentified species resulted in closures in this area during the month of August.

4.1.2 Denmark

DSP toxicity was detected in blue mussels (*Mytilus edulis*) in the outer part of the Danish Wadden Sea in August 2001. The causative organism was *Dinophysis acuminata* (1.3×10^3 cells l^{-1}). Domoic acid was detected in shellfish from the Limfjord at low concentration (20 µg kg^{-1}) during a major bloom comprised of several species of *Pseudo-nitzschia*. There were no reports of human intoxication caused by consumption of Danish shellfish in 2001.

A bloom of *Chattonella* spp. was observed during March-April, primarily in the Kattegat and coastal North Sea. No evident harmful effects were associated with this bloom. *Karenia mikimotoi* was also observed in moderate concentrations during late summer, but no harmful effects were observed to be related to the bloom. During the summer, several spectacular blooms of the cyanobacterium *Nodularia spumigena* developed in the Western Baltic, in the Kattegat/Belt Sea/Øresund area. The bloom resulted in recommendations from the authorities that the public should stay out of the water, if it was discoloured. The effect of the bloom on the recreational use of coastal areas as well as on the tourist industry is not known.

4.1.3 Germany

4.1.3.1 North Sea

The non-toxic nuisance species *Noctiluca scintillans* and *Phaeocystis globosa*, as well as the symbiont- bearing ciliate *Mesodinium rubrum* (Synonym: *Myrionecta rubrum*) caused water discolouration at the sea surface during the year, primarily due to foam production. Adverse effects such as oxygen depletion were not noticed. *Dinophysis acuminata* was recorded in the East Friesian Wadden Sea (ICES Region IVB) and mussels from this region were found to contain DSP toxin concentrations at roughly the maximum permitted level. Mussel harvesting was closed at the end of September, 2001 at some of the mussel culture sites. Other toxic or potentially toxic phytoplankton species were recorded in low numbers at various times of the year, but they did not cause any problems. These species included the dinoflagellates, *Alexandrium tamarense* and *A. ostenfeldii*, the diatom *Pseudo-nitzschia* sp. and the raphidophytes *Fibrocapsa japonica*, *Chattonella verruculosa* and *Heterosigma akashiwo*.

4.1.3.2 Baltic Sea

In German coastal waters of the Baltic Sea, cyanoprokaryonta ("blue-green algae") were abundant. During routine monitoring, *Nodularia spumigena* was present in high numbers and about 13.5 µg nodularin was detected in a water sample of about 50 ml. Recreational beaches and bathing were partly closed at the end of July, 2001 along the coast of Schleswig-Holstein. Satellite images showed a large accumulation of these algae in the Baltic Sea, in particular, in Danish waters and off Mecklenburg-Vorpommern, Germany. As a result of wind driven accumulation of these cyanobacterial cells, the beaches of Schleswig-Holstein were closed. A strong discoloration of the inner part of Kiel fjord was caused by up to 3.0×10^6 cells l^{-1} of the dinoflagellate *Prorocentrum minimum*. The potentially toxic diatom *Pseudo-nitzschia pungens* or *P. multiseries* (no EM-determination) was present in some samples, but no adverse effects were noted.

4.1.4 Ireland

As in previous years, an extensive monitoring effort resulted in the detection of DSP, AZP and ASP toxins in shellfish. This necessitated the closure of shellfish production areas for periods of, in some cases, up to 10 months. No major mortalities of either caged fish or other marine organisms were observed. The highest detected concentration of *Karenia*

mikimotoi cells was 7.0 X 10^3 cells l^{-1} in Lough Swilly in July. There were no reports of discoloured waters during this time.

From a total of 2,316 phytoplankton samples analysed, 758 occurrences of *Dinophysis* spp., including *D. acuminata* at concentrations up to 6.0 X 10^3 cells l^{-1} and *D. acuta* up to 1.0 X 10^3 cells l^{-1} were recorded. Species of *Alexandrium* were detected in 246 samples at concentrations up to 9.4 X 10^4 cells l^{-1}.

Samples of sediment were collected along the South West coast to look for cysts of toxic species. Cysts of *Lingulodinium polyedra* were isolated and germinated. Samples of these were analysed with the cooperation of the Veterinary Institute in Oslo and found to contain yessotoxin at levels of 0.3 pg $cell^{-1}$, suggesting that 2 x10^5 cells would be required to toxicify mussels to the threshold level of 100 µg g^{-1}.

An extensive bloom of *Alexandrium minutum* (up to 9.4 X 10^4 cells l^{-1}) accompanied by lower concentrations of *A. tamarense*, was observed along the West Coast in early August. PSP toxicity was not detected in shellfish at this time. Such high concentrations of *A. minutum* had not been previously recorded in Ireland, but this event coincided with a similar bloom recorded by FRS Laboratory, Aberdeen to the West of the Outer Hebrides.

Domoic acid above 20 µg g^{-1} was found in scallops, mainly in hepatopancreas tissue (84% of samples analysed), with lower levels (<8.1% over 20 µg g^{-1}) found in the soft tissues typically consumed (adductor muscle and gonad). The causative genus is presumed to be *Pseudo-nitzschia*, which was common in all areas around Ireland at concentrations up to 7.2.X 10^5 (*P. delicatissima* group). The reason domoic acid was only detected in scallops has not been determined.

4.1.5 Latvia

No harmful algal blooms were observed in Latvian territorial waters in 2001. This could be explained by the relatively windy weather that occurred during the summer. The highest abundance of the principal harmful species typically found (*Nodularia spumigena, Aphanizomenon* spp*., Dinophysis acuminata*, *and Chrysochromulina* spp.) occurred in the Gulf of Riga during the summer (June – August). High numbers of *Chaetoceros danicus,* maximal concentration (8.3 X 10^4 cells l^{-1}), were observed in November. The concentration of potentially toxic cyanobacteria, *N. spumigena and Aphanizomenon* spp. did not exceed 3.2 X 10^3 and 6.7 X 10^4 filaments l^{-1}, respectively. The DSP toxin-producing species, *Dinophysis acuminata* reached 2.8 X 10^3 cells l^{-1}, and *Chrysochromulina* spp. achieved only 5.5 X 10^5 cells l^{-1}. This year there were no reports of harmful events.

4.1.6 Netherlands

A *Phaeocystis globosa* spring bloom was monitored in the coastal area off Zeeland (Voordelta). This bloom lasted for over a month, with maximal concentrations of 2.0 to 7.5 X 10^7 cells l^{-1} recorded on 11 May 2001. In the saline, oligotrophic Lake Grevelingen, the influx of *Phaeocystis* from the Voordelta through the Brouwerssluis led to an unprecedented rapid oxygen depletion and hydrogen sulphide formation near the bottom. Dr M. de Kluyver (University of Amsterdam) will report the extent of the damage to marine fauna in 2002.

In early May, mortalities of shellfish (mussels) were reported from the western part of the Oosterschelde, an estuary south of Lake Grevelingen. The RIKZ hypothesized that the shellfish had been killed by low oxygen concentrations due to sedimentation of *Phaeocystis* cells.

To the north of the Rhine-Meuse estuary, in the outer harbour of Ijmuiden, health complaints were registered by the crew of the dredger clearing low density sediment from the mouth of the harbour. Measurements showed high hydrogen sulphide levels in the ship, especially in the hold where the dredged sediment was stored, and dredging had to be postponed for two weeks. As *Phaeocystis* bloomed from April 10 to May 17 at a nearby monitoring site, and reached concentrations up to 1.8 X 10^7 cells l^{-1}, it is probable that the low density, hydrogen sulphide producing 'sediment' in the outer Ijmuiden harbour was the result of a settled *Phaeocystis* bloom.

In August, swimmers at the 'Hoek van Holland' beach (in the Rhine outflow) reported skin irritations. Microscopic counts of a sample collected at this time indicated a *Phaeocystis* concentration of 4.0 X 10^9 cells l^{-1}.

At the beginning of August, the water column in the Dutch coastal zone stratified due to freshwater input originating from the Rhine. The concentration of *Dinophysis acuminata* at monitoring location Nw10 was 3.2 X 10^3 cells l^{-1} in the surface layer and 1.2 X 10^3 cells l^{-1} in the bottom layer. In the Wadden Sea, the RIVO observed *D. acuminata* cells from the end of August to September at concentrations ranging from 1.0 X 10^2 to 3.5 X 10^3 cells l^{-1}. Rat bioassays of

hepatopancreas of the blue mussel (*Mytilus edulis*) were negative. HPLC analysis revealed the presence of 100 µg $100g^{-1}$ okadaic acid, which is below the safety limit. DSP toxicity was not reported.

4.1.7 Norway

In 2001, occurrences of DSP toxicity were ”normal”, with some problems along the southern coast of Norway and in the large fjords along the west coast, increasing from the outer to the inner parts of the fjords. The problems with PSP toxins in shellfish were less than normal, with hardly any records above regulation level. For the first time, YTXs and AZAs were confirmed at levels resulting in banning of mussel harvesting for a period (see more under ToR “Emerging Toxins” and “New Findings”). A bloom of *Chattonella* sp. (cf. *C. marina*) in March 2001 killed about 1,100 tonnes of salmon along the southern coast of Norway. The bloom occurred in cold and somewhat low-salinity surface waters. For the first time, *Pfiesteria* has been recorded in northern Europe, from a location in the inner Oslofjord (see more under “New Findings”).

4.1.8 Scotland

During May and June 2001, high numbers of cells of *Alexandrium* spp. were again recorded along the east coast of Scotland, and also in the Orkney and Shetland Islands. This was associated with high levels of PSP toxicity in mussels (*Mytilus edulis*). PSP toxicity was also recorded in *M. edulis* from sites on the west coast, and a bloom of a small *Alexandrium* species was recorded in the Outer Hebrides in July. This was associated with high levels of the PSP toxin in *M. edulis* from this area. *Dinophysis* cells were routinely recorded in Scottish coastal waters, with numbers peaking during July and August. Extensive voluntary closure agreements (VCAs) were implemented from May–October because of positive bioassay results for DSP toxicity in *M. edulis*. In contrast to previous years when *D. acuminata* and *D. acuta* dominated the phytoplankton, *D. norvegica* was also present in large numbers this year.

Pseudo-nitzschia spp were routinely found in water samples although frequently at levels less then 5.0×10^4 cells l^{-1}. ASP toxin was again found at low levels in *Mytilus edulis*. However, domoic acid levels in *Pecten maximus* gonads at >20 µg $100g^{-1}$ were recorded from numerous offshore scallop-fishing boxes from along the west coast of Scotland and the Orkney Islands.

A number of algal blooms caused mortalities of caged fish during 2001. A bloom during late May, comprising mainly *Heterocapsa triquetra* at a density of 1.0×10^6 cells l^{-1} killed farmed fish in the Shetland Islands. A second bloom, which caused substantial financial losses to fish farmers in the Orkney and Shetland Islands occurred at the end of August and was composed of *Gymnodinium* spp. recorded at a concentration of roughly 9.0×10^6 cells l^{-1}.

4.1.9 Spain

The problems caused by harmful algae in the Galician and Andalusian communities of Spain are mainly due to toxic species that even at low concentrations can render shellfish unsuitable for human consumption. In Cataluña (Mediterranean coast), the main problem is with bloom-forming species causing water discolouration that affects summer tourism.

4.1.9.1 Andalucía

On the Western Atlantic side of Andalucía, there were very persistent occurrences of DSP toxin associated with *Dinophysis acuminata* that lasted for three months in the spring and two months in summer. On the Eastern Mediterranean coast, *Gymnodinium catenatum* and PSP toxins were reported in summer (June-July) and autumn. There were also ASP toxin outbreaks in winter (January-February) caused by *Pseudo-nitzschia* spp. and mainly affecting the marketing of scallops.

4.1.9.2 Cataluña

Blooms of *Alexandrium catenella,* first recorded in the area in 1996 have become a recurrent event. Dense blooms were recorded in Tarragona harbour in May, and moderate populations in the Ebro Delta in October-November rendered bivalves toxic above regulation levels. Also, high concentrations of *A. minutum*, *Dinophysis sacculus* and *Pseudo-nitzschia* spp. were found in June. High biomass blooms of non-toxic species (*Gymnodinium impudicum, A. taylorii, Calyptrosphaera sphaeroidea)* occurred in August, causing social alarm and negatively affecting the tourist industry.

4.1.9.3 Galicia

Dinophysis acuminata in summer and *D. acuminata* and *D. acuta* in the autumn resulted in prolonged closures of bivalve marketing caused by DSP toxicity in the Galician Rías Bajas. *Pseudo-nitzschia australis,* the causative organism of ASP toxicity, caused closures in September-October. Water discolorations caused by proliferation of *Noctiluca scintillans* in summer*,* and *Prorocentrum minimum* in October created social alarm. For the first time in the area, a bloom of *Karenia mikimotoi* was recorded in October, but it did not cause any damage to either cultured or wild shellfish populations.

4.1.10 Sweden

4.1.10.1 Skagerrak and Kattegat

Harmful species recorded included *Chattonella* spp., *Karenia mikimotoi, Dinophysis* spp. and *Alexandrium* spp.

A bloom of the fish-killer *Chattonella* spp. followed the normal diatom spring bloom. The bloom was first detected on March 1, 2001 at a location close to the mouth of river Nordre Älv. At the end of March, the bloom was distributed over large parts of the Kattegat and the Skagerrak and could be followed using satellite derived data (SeaWIFS-sensor) during cloud-free conditions. The bloom disappeared during the last days of March when there was a change in the weather towards stronger winds. There was a shift in the cell-size of *Chattonella* sp. towards cells <10 µm during the bloom. These mini-cells dominated when the highest abundance of *ca*. 1.2 X 10^7 cells l^{-1} was observed. No harmful effects were observed in Sweden; however, deaths of caged fish (1000 tons of salmon) were reported from Norway. This was the third recorded bloom of *Chattonella* in the area. The first occurred in 1998 and the second in 2000. Both of these previous blooms occurred in May-June. Re-analysis of samples from previous years showed that the species was present at the Swedish west coast (Lysekil) in 1993.

In August, the fish-killing dinoflagellate, *Karenia mikimotoi* was observed in the Skagerrak. The cell abundance was low and no harmful effects were observed. Concentrations of DSP toxins in blue mussels were above the limits for harvest along the Swedish Skagerrak coast and the northern part of the Kattegat coast all year, except from mid-April to mid-July. The highest concentrations of DSP toxins, *ca*. 700 µg kg^{-1} mussel meat, were observed in November. This is lower than in 2000, when the maximum was 1900 µg kg^{-1}. Low numbers of the PSP toxin-producing species *Alexandrium tamarense* were observed during the year.

4.1.10.2 Baltic Proper

Harmful species recorded included *Nodularia spumigena.* Substantial blooms of large cyanobacterial cells were observed in the Baltic from the beginning of July to the beginning of August. North of the island of Gotland, the non-toxic species, *Aphanizomenon* sp. dominated. South of Gotland, the toxic species, *N. spumigena* was dominant and occurred in very high abundance. Satellite images showed strong surface accumulations south and southeast of the island of Öland. Tourism was affected since swimmers were advised not to go into the water. Furthermore, the public found the cyanobacteria to be a nuisance. The reporter is aware of no toxic effects but no toxin measurements were made.

4.1.10.3 Bothnian Bay

No report of harmful algal blooms in year 2001.

4.1.11 USA

2001 was basically a “normal” year for HABs in the U.S. As happens most years, PSP toxicity was recorded in the New England states, as well as on the West Coast in California, Oregon, Washington and Alaska. In western Maine, there was very little shellfish toxicity, following a year with the highest levels in 10 years. Conversely, eastern Maine experienced the most widespread PSP toxicity of the past several years, following a year with almost no toxicity. ASP toxin was recorded in California, Oregon, and Washington. An area of New Jersey that has had brown-tides experienced a significant event again this year. New York State also experienced a brown-tide, along south-shore estuaries. The Florida red tide caused by *Karenia brevis* occurred again in 2001, including along the southwest coast of Florida, even down to the Florida Keys, causing fish kills and respiratory irritation. This year there were no reports of fish kills or fish lesions definitively attributed to *Pfiesteria* in North Carolina or Chesapeake Bay.

4.2 Decadal Maps

The format and content of the decadal maps were discussed at length and the following recommendations were proposed:

1) The map on ciguatera toxins should be deleted.
2) In addition to the decadal maps, annual maps should be produced, allowing annual trends to be followed.
3) The maps presenting "Presence of toxins in X" should be renamed to "Presence of toxins above limits for closure of shellfish harvesting in X".
4) The maps presenting "Presence of XXX toxins" should be renamed to "Presence of XXX toxins above limits for closure of shellfish harvesting".
5) A label for yellow = non-ICES countries should be added to the map legends.
6) A map for "Presence of yessotoxins above limits for closure of shellfish harvesting" should be added.
7) The map for "Animal and plant mortalities" should be renamed to " Mortalities of marine animals and plants".
8) The map "Other toxic effects - cyanobacteria" should be renamed to "Other harmful events – cyanobacteria" and changed to reflect only harmful algal events. This means that offshore blooms should not be included on these maps; however, warnings for recreational use of the water due to high biomass of cyanobacteria should appear.
9) A new map to be named "Other harmful events" should be added. This map would include events such as masses of foam on beaches, very high abundance of *Noctiluca*, oxygen depletion due to high biomass blooms, etc.

4.3 HAEDAT Database and Harmful Event Report Form

HAEDAT is the on-line IOC-ICES Harmful Algae Event database available at the IOC web site: http://ioc.unesco.org/hab/data3.htm#1 and that contains National Reports from ICES countries from 1987 to 1998. Data from 1999–2000 have been entered but are not available on line.

With a view that HAEDAT will become a global harmful event database, preliminary agreements to joint HAEDAT have just been reached with both IOC regional groups: ANCA and FANSA. These agreements are the continuation of the expansion work started last year with PICES countries.

Monica Lion (IOC-IEO SCCHA, Spain) reported on the analysis of the data in the HAEDAT database. A number of difficulties were highlighted concerning the data entered on the HAE-DAT forms. This was mainly due to inconsistencies in the data entered. The reporting format for the data varied from country to country and required a lot of proofing by the database administrator prior to being entered. This variability in data entry restricted the ability of investigators to search the database using certain fields. Examples of this variability include: misuse of species synonyms; inconsistency in entering the exact latitude and longitude, or the description of the location given in the 'comments' box; variation in toxin assay data reported from different methods; and variation among countries as to whether or not the shellfish toxicity box should be "ticked" when shellfish were harmful for human consumption versus when the shellfish themselves were directly affected by the toxin. Some countries had supplied a report form for each toxin event recorded, whereas others had condensed these events and reported toxin events within a region.

Two Terms of Reference are proposed for next year to identify inconsistencies in submitted HAEDAT forms and to examine the possibility of creating HAEDAT maps directly from the database.

5 GEOHAB

Term of Reference b: Review GEOHAB implementation in the ICES area.

5.1 GEOHAB: ICES-IOC-SCOR SGGIB Study Group on GEOHAB Implementation in the Baltic

Bengt Karlson (Sweden) reported on the meeting of the ICES-IOC-SCOR SGGIB (Study Group on GEOHAB Implementation in the Baltic). This Study Group (members: Kaisa Kononen (Finland), Bengt Karlson (Sweden), Edna Granéli (Sweden) and Maija Balode (Latvia)) met in Stockholm on November 24, 2001 and compiled a proposal for a co-operative HAB study with six sub-programmes in the Baltic Sea. Alternatives for funding of the study were discussed. An invited meeting to prepare a proposal for the European Commission concerning Programme Activity 3. *Hydrodynamical Control of HAB Development*, and Activity 6. *Modelling* is planned. The World Bank GEF (Global Environment Facility) Project will fund a 'Ship of opportunity' programme during autumn 2002 (*pers. comm*., Jan Thulin, ICES, 4 March 2002) that will be part of a Baltic GEOHAB study. During the Baltic Sea Science Congress in

Stockholm, an open workshop about the Baltic GEOHAB initiative was held. About 40 people attended the workshop, indicating strong interest in the project. The Chair of the SGGIB, Kaisa Kononen, has resigned.

5.2 C-GEOHAB

Jennifer Martin (Canada) reported on the progress of GEOHAB in Canada. A preliminary one-day meeting was held immediately following the 7th Canadian Workshop on Harmful Marine Algae in May 2001 to discuss the level of interest and Canadian involvement. A decision was made to hold a Workshop (organized by Fisheries and Oceans Canada) in Montreal from 19–20 October 2001. The Workshop was chaired by Bjorn Sundby (U. du Québec), and organized with the intent of coordinating scientific research and cooperation in order to develop international capabilities for assessment, prediction and mitigation of HABs through a better understanding of the ecology and oceanography affecting these algae. The Workshop was an Open Science Meeting with 30 participants that included a representative from the Scientific Steering Committee of the International GEOHAB Programme (Allan Cembella) and participation from universities, government departments and agencies, industry and other interested scientists. A special effort was made to recruit new scientists from disciplines not always associated with the HAB field, such as virus experts, optical oceanographers, physical oceanographers, and geochemists. A need was clearly expressed for coordinated studies of population dynamics of HABs.

A title for the Canadian GEOHAB programme was proposed: "Population Dynamics of Canadian HABs (*Heterosigma*, *Alexandrium* and *Pseudo-nitzschia*)". Three themes were outlined: 1) population dynamics of key HABs in comparable ecosystems; 2) biologically mediated growth and loss processes; and 3) novel observation systems and data analyses towards improved prediction. Theme 1 will include physical/chemical oceanography, HAB growth processes, nutrient uptake, vertical migration, cyst-related processes and loss processes. Theme 2 will include grazing-related losses (pelagic and benthic grazers, allelochemical interactions, the influence of fungi, bacteria and viral pathogens, sedimentation and bio-deposition, and sediment geochemistry), as they relate to vegetative cells and cysts. Theme 3 will include continued development of novel observation and detection methods, enhanced capability for long-term and synoptic observation, novel data analyses using retrospective and historical databases, and development of assimilation forecasting models for application in coastal observation systems to improve prediction. A technical report summarizing the Montreal Workshop has been published (Martin, 2002).

Future plans include holding a Planning Workshop in Montreal in August 2002 to: focus the efforts of Canadian scientists interested in HABs; consolidate the efforts into the production of an explicit Canadian GEOHAB Science Plan; follow the recommended research themes of the international GEOHAB programme with international collaborators; submit the Canadian Science Plan to the international GEOHAB Scientific Steering Committee; and to seek funding for the realization of the proposed work by preparing grant requests.

5.3 Reference

Martin, J. L. 2002. Developments for a Canadian GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms) Program: 2001 Workshop Report. *Can. Tech. Rep. Fish. Aquat. Sci.* 2400: vii + 44 p.

5.4 CEOHAB

Don Anderson (USA) reviewed progress towards creation of HAB projects in Chinese coastal waters. The distinction between international and national HAB research programmes is important here. China recently awarded significant long-term (five-year) funding for CEOHAB – the Chinese Ecology and Oceanography of Harmful Algal Blooms programme. This is, however, a national research programme that will provide support for an array of research teams within China to study HAB problems in three different regions – the Bohai Sea, the East China Sea, and the South China Sea. Although some non-Chinese scientists may join these CEOHAB programmes as advisors or participants, this is not viewed as a core GEOHAB programme at this stage. To create such a programme, a workshop should be convened to discuss the creation of a multi-national project focusing on one of several topics that might best be addressed in Chinese waters.

The WG discussed this issue extensively, recognizing that the significant and growing eutrophication problem along the Chinese coast provides a unique opportunity for the global HAB community to investigate high biomass, nutrient-driven bloom phenomena. One possible site for such a study would be the Bohai Sea, which is the site of massive red tides that appear to be linked to heavy pollution loading and significantly reduced freshwater inputs due to drought or land-use activities. Likewise, China has several large rivers that enter the ocean carrying both high nutrient loads and high levels of suspended sediment. The unique dynamics of the HABs in these regions have great relevance to blooms that occur in other parts of the world with similar hydrographic, environmental, and cultural characteristics. For example, anticipated changes in the flow and suspended sediment characteristics of the Yangtze River in the coming

years due to the Three Gorges Dam project suggests a rich opportunity to investigate and explain the changes that will likely occur in plankton dynamics in the receiving waters of the East China Sea due to alterations in N:P, N:Si and other nutrient ratios.

The Working Group recommends that the GEOHAB Scientific Steering Committee take an active role in formulating and eventually funding an international project dealing with high biomass blooms along the Chinese coast. To accomplish this, the SSC should consider providing financial and organizational support for an international workshop on eutrophication and high biomass HABs, the goals of which might be to identify: 1) key scientific unknowns and hypotheses; 2) regions of the world where such issues are best addressed; 3) the general research elements needed in a research programme to investigate these phenomena; 4) potential funding sources, including international agencies; and 5) the next steps needed to maintain progress towards implementation of a GEOHAB programme on this topic.

5.5 LIFEHAB

Beatriz Reguera (Spain) reported on the LIFEHAB workshop. The understanding and quantification of processes involved in the life cycle of HAB species is a fundamental step in building reliable conceptual and predictive models that can be effective tools in HAB management and mitigation. Information available about life cycle strategies of harmful species is fragmentary, and improvements in basic knowledge are needed to estimate the impact of different life history stages in the population dynamics of HABs.

The LIFEHAB Workshop supported by the Commission of the European Union as part of the EUROHAB initiative (European Initiative on Harmful Algal Blooms) was organised to create a forum of discussion among specialists from different disciplines (taxonomy, physiology, biological oceanography, molecular biology, modelling) related to different groups of harmful algae (diatoms, dinoflagellates, haptophytes, raphidophytes). The objectives of the meeting were to:

1) summarise current knowledge on the life history of harmful species,
2) identify the main gaps of knowledge,
3) discuss the most appropriate approaches and methods to address the role of life cycles in HAB dynamics.

Twenty-one participants from 14 countries met in Calviá (Palma de Mallorca) from 24–27 October in Mallorca (Balearic Islands, Spain). Participants presented contributions that were followed by group discussions and recommendations. The collection of keynote presentations, communications, group discussion consensus, recommendations, and a bibliography were disseminated to the participants through a web page. When these proceedings are completely edited, they will be made public on the Internet. A small book will be published in the series “Research in Enclosed Seas” of the European Commission (Key Action: Energy, Environment and Sustainable Development). The content index is shown in Annex 3.

6 NEW AND EMERGING TOXINS

Term of Reference c: Review existing data on the identification, distribution and toxicological significance of new and emerging phycotoxins and causative organisms, in terms of human health significance, HAB population dynamics, and effects on marine food webs.

Reported by Bernd Luckas (Germany) and Allan Cembella (Canada) with assistance from Michael Quilliam (Canada) and Alexander Ruehl (Germany).

6.1 Background

The problems caused by new and emerging phycotoxins in the ICES region have assumed greater importance to regulatory and public health agencies, the aquaculture and wild fisheries industry and seafood consumers within the past decade. Heightened awareness within the research community, coupled with increased research and monitoring has led to the discovery of new groups of phycotoxins and to the structural elucidation of a plethora of novel analogues among the previously known toxin types. A few examples of emerging toxins now known to occur within the ICES region in plankton and/or shellfish are illustrated in Figure 1. These accomplishments in toxin detection and quantitation have been made possible by improvements in analytical technologies (LC-MS, LC-FD, etc.) and toxin-specific assays (radio-receptor-, immuno-, cytotoxicity-assays). In turn, this work has been dependent upon an increasing but still incomplete supply of analytical standards and reference materials for instrument calibration and method validation.

The definition of an "emerging" phycotoxin is rather imprecise and largely operational (Cembella *et al.,* 2002). The WG considered several alternative but not necessarily mutually exclusive descriptions of emerging toxins:

- known toxins in atypical organisms or habitats (e.g., amnesic shellfish poisoning [ASP] toxins found in diatoms and shellfish from northern Europe; neurotoxic shellfish poisoning [NSP] toxins in New Zealand; paralytic shellfish poisoning [PSP] toxins in abalone);
- novel toxin groups (e.g., azaspiracid [AZA], gymnodimine);
- new derivatives within known toxin groups (e.g., dinophysistoxin-4 [DTX4], pectenotoxin-2 seco-acid [PTX2sa]);
- toxins of known origin but unknown or poorly defined toxicity and pharmacology (e.g., spirolides, yessotoxin [YTX]);
- toxins of suspected but unproven algal origin or unknown causative organism (e.g., palytoxin, pinnatoxin)

These novel or emerging phycotoxins vary widely in structural complexity, polarity, chemical stability, and potency in mammalian systems, and phylogenetic and biogeographical distribution among marine microalgae. For comparison, relative potency of several of the lipophilic phycotoxins found in the ICES region is shown in Table 1.

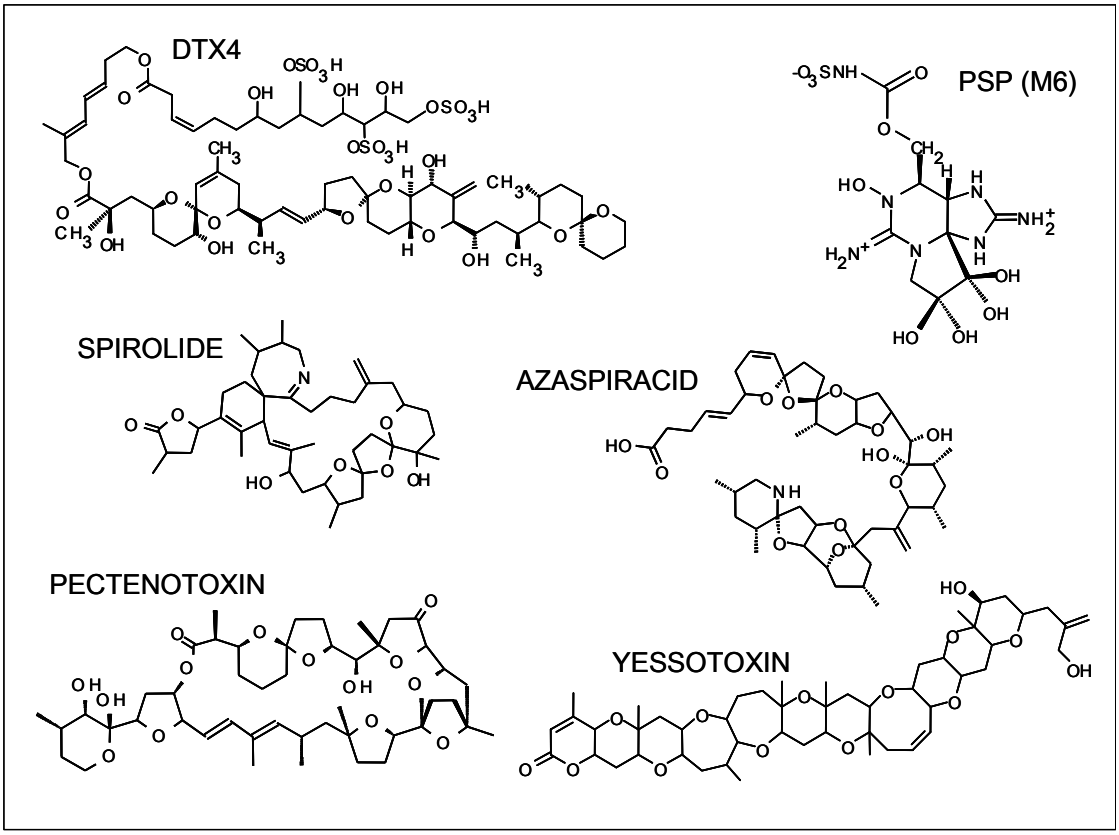

Figure 1. Examples of emerging phycotoxins that have been identified in plankton and/or shellfish from the ICES region in recent years. Note that these analogues shown are only representative of a particular toxin group and that multiple derivatives of each toxin type are frequently present.

6.2 Azaspiracids

In November 1995, a severe outbreak of shellfish poisoning occurred in the Netherlands, after consumption of mussels from Ireland. In humans, the symptoms - nausea, vomiting, severe diarrhoea – were considered to be "DSP-like", but chemical analysis did not indicate the presence of significant levels of such compounds in the shellfish. A new group of marine toxins, named azaspiracid (AZA), was subsequently isolated and structurally elucidated from mussel tissues collected from Killary Harbour, Ireland. Two azaspiracid analogues (AZA2 and AZA3) were recently isolated from Irish mussels, and even more derivatives have now been described. Since the original toxicity incident, AZA toxin has been found in shellfish from the UK and Norway.

Table 1. Acute toxicity (LD_{50}) of various lipophilic phycotoxins after i.p. injection into mice. Only those lipophilic toxins found in shellfish and the corresponding toxigenic microalgae are included. *In the case of PTX and YTX groups, oral toxicity and cytotoxicity studies have been carried out only for PTX1, PTX2 and YTX. For other analogues, the pathology is likely to be similar or identical to that of the parent compounds, but is currently undefined. (adapted from Fernández, Richard and Cembella, in press; references cited therein)

Toxin Group	Analogue	Toxicity (μg kg^{-1})	Pathology
Okadaic acid	OA	200	diarrhoea; tumour promotion
Dinophysistoxin	DTX1	160	diarrhoea
	DTX3	500	diarrhoea
Pectenotoxin	PTX1	250	hepatotoxic
	PTX2	230	hepatotoxic; diarrhoea
	PTX3	350	*
	PTX4	770	*
	PTX6	500	*
	PTX7	>5000	*
	PTX8	>5000	*
	PTX9	>5000	*
Yessotoxin	YTX	100	cardiotoxic
	HydroxyYTX	500	*
	TrinorYTX	220	*
	HomoYTX	100	*
	45-hydroxyhomoYTX	500	*
	DesulfoYTX	500	*
	CarboxyYTX	500	*
Azaspiracid	AZA	200	diarrhoea
	AZA2	110	diarrhoea
	AZA3	140	diarrhoea
	AZA4	470	diarrhoea
	AZA5	1000	diarrhoea
Gymnodimine		96	unconfirmed
Brevetoxin	BTX-B1	50	neurological
	BTX-B2	300	neurological
	BTX-B3	>300	neurological
Spirolide	B	200	unconfirmed
	des-methyl-C	40	unconfirmed

Azaspiracids are characterized by the presence of novel five- and six-membered spiro-rings, one of which contains nitrogen. Since AZA toxins are structurally and toxicologically different from previously known toxins, this new shellfish toxin syndrome has been called azaspiracid poisoning (AZP). Analytical methods involving liquid chromatography – mass spectrometry (LC-MS) have been developed for the analysis of AZA in shellfish. Such LC-MS methods are highly specific and sensitive and are readily automated for shellfish toxin monitoring.

The etiology of AZP is unknown and no unusual phytoplankton species were observed during any of the AZP toxic episodes. However, detailed studies were not undertaken until some time after human poisonings were reported and because of the persistence of these toxins, the events that led to toxin accumulation in shellfish could have occurred much earlier. Nevertheless, the pattern of methylation in AZA is typical of toxins produced by dinoflagellates. Some recent evidence involving micropipette isolation of plankton cells followed by LC-MS analysis has suggested that AZA may be associated with the dinoflagellate *Protoperidinium* spp., and more specifically with *P. crassipes*, although the evidence is not conclusive.

6.3 Spirolides

The term “spirolide” describes a group of biologically active compounds first isolated in 1995 from the digestive glands of shellfish from Nova Scotia in eastern Canada (Hu *et al.,* 1995a). Spirolides are so-called “fast-acting toxins” causing death with characteristic neurotoxic symptoms within several minutes when injected intraperitoneally (i.p.) into mice (Richard *et al.,* 2002). Later, spirolides were also found in plankton samples from coastal waters of Nova Scotia (Cembella *et al.,* 2000). With molecular weights ranging from 691 to 711 and a macro-cyclic structure, spirolides belong to a broad grouping of polyether toxins, which also includes the okadaic acid group, brevetoxins (BTX), ciguatoxins (CTX), pectenotoxins (PTX), and yessotoxins YTX). The existence of toxin pairs with a molecular weight difference of 2 Da related to one double bond is typical for spirolides (-A/B, -C/D, and -E/F). Two new structural variants were recently discovered in plankton; these des-methyl derivatives are formed by demethylation of spirolide C and spirolide D, respectively.

The origin of spirolides remained cryptic until recently, when the annual recurrence was related to the marine dinoflagellate *Alexandrium ostenfeldii* (Cembella *et al.,* 2000). *Alexandrium ostenfeldii* is morphologically closely related to *A. tamarense* and *A. fundyense*, which are well-known producers of paralytic shellfish poisoning (PSP) toxins. However, the strains of *A. ostenfeldii* isolated from Nova Scotia, Canada, which are responsible for the occurrence of spirolides in this region, do not produce PSP toxins. On the other hand, certain *A. ostenfeldii* strains isolated from Limfjord, Denmark may produce both toxin groups. Thus far, only *A. ostenfeldii* has been identified as spirolide producer, but the possibility that other taxa might also be capable of spirolide synthesis cannot be excluded.

At Graves Shoal and Ship Harbour, two important shellfish aquaculture locations in Nova Scotia, annually recurring blooms of spirolide-producing microalgae have been monitored since 1996. The toxin profiles found at these locations revealed remarkable differences. In natural phytoplankton from Graves Shoal, spirolides B and D dominate, whereas spirolides A and C are minor components. In contrast, the profile of spirolides found at Ship Harbour is dominated by the demethylated derivative of spirolide C. Recent analysis of natural plankton assemblages containing *A. ostenfeldii* from the Gulf of Maine, USA has revealed the presence of spirolides with a profile similar to that of Graves Shoal populations (A. Cembella and D.M. Anderson, unpubl. data).

Monitoring programs in North America and Europe do not routinely monitor for the presence of spirolides. Furthermore, spirolides cause confusing symptoms in the lipophilic assay for DSP toxins, and are normally excluded from aqueous extract of PSP toxins for the AOAC mouse bioassay, therefore they can be easily overlooked during routine bioassays.

To determine the extent of spirolide distribution in European waters, analytical tools for their identification and determination were implemented. An LC-MS method allowing for the simultaneous determination of different algal toxins including spirolides was developed. This method was applied to bulk plankton samples on board the research vessel “Heincke” (Germany) during the research cruise to areas along the east coast of Scotland in May 2000. As the volume of water sampled with the plankton net was not recorded in the study, only qualitative results indicating the presence or absence of spirolides were obtained. Nevertheless, it was obvious that a large area of the Scottish east coast was affected by spirolides. The occurrence of *A. ostenfeldii* with spirolides at all stations provides strong circumstantial evidence that this is also the causative organism in European waters, as in Nova Scotia, Canada. However, the different spirolide profiles observed in samples from the Scottish east coast appears to indicate that different strains are present. In the sampling procedure, a large quantity of seawater was filtered with a plankton net, thus trapping organisms from larger size distributions and higher trophic levels. Little is known about the fate of spirolides once metabolised by grazers and this requires further study.

6.4 Yessotoxin

Yessotoxin (YTX) was first identified after a shellfish toxicity incident in Japan associated with the consumption of Japanese scallops (*Patinopecten yessoensis*) in the mid-1980s. Initially, YTX toxicity was first believed to be part of “DSP toxicity” but the syndrome does not cause diarrhoea. The toxic symptoms are not well defined in humans, but YTXs are cardiotoxic in rodents. Acute symptoms following the injection of YTX into mice resemble those typical of neurotoxins and are characterized by short survival time, with convulsions and jumping before death (Aune *et al.,* 2002). For these reasons, an involvement of the nervous system in YTX toxicity was originally presumed. The hypothesis was also strengthened by the resemblance of the YTX structure to that of brevetoxins (BTXs) and ciguatoxins (CTXs), for which there exists pharmacological evidence for the induction of an increase in cell membrane permeability to sodium ions. Because of its frequent coexistence with dinophysistoxins (DTXs) and pectenotoxins (PTXs), YTX was tentatively included in the DSP toxin category. However, the YTXs are less toxic than okadiac acid (OA), DTXs and PTXs by oral administration. Furthermore, YTX also differs from DSP toxins in etiology; YTX is produced by the dinoflagellate *Protoceratium reticulatum*, whereas homoYTX has only been identified in *Lingulodinium polyedrum* (= *Gonyaulax polyedra*).

Yessotoxins have 11 contiguously transfused ether rings and an unsaturated side chain. The base compound, YTX has a backbone of 47 carbons, a terminal side chain of nine carbons, and two sulphate esters, and it lacks carbonyl groups. The first naturally occurring YTX analogue was identified in the Japanese scallop, *Patinopecten yessoensis* as 45-hydroxy YTX. The occurrence of toxicity in the mussel *Mytilus galloprovincialis* from the northern Adriatic Sea, which contained only low amounts of OA and no YTX, led to the discovery of homoYTX and 45-hydroxyhomoYTX.

For accurate characterization of YTXs, a selected reaction monitoring (SRM) LC/MS-MS method has been developed. This method is based on a simple acetone extraction of YTXs from shellfish followed by LC/MS-MS using aqueous acetonitrile containing ammonium acetate as the mobile phase. The method was shown to be specific, sensitive, and rapid for the determination of YTXs in shellfish and phytoplankton. This method has also unambiguously demonstrated false-negative results of the mouse bioassay for a number of mussel samples.

In recent years, research on the occurrence of YTX in shellfish from different marine areas has indicated that YTX contamination of molluscs is more important and widespread than previously thought. Unfortunately, the toxicity pattern is frequently complicated by the simultaneous presence of true DSP toxins in shellfish. For example, from March to November 1997, monthly concentrations of DSP toxins and YTX in mussels were determined from nine locations from the coast to the inner part of the Sognefjord, Norway (Ramstad *et al.,* 2001). Concentrations of both DSP toxins and YTX in mussels increased with distance from the coast, although the number of *Dinophysis* cells (associated with DSP toxins) was low. However, an increasing number of *D. acuta* cells were registered in the fjord beginning in July and this was associated with higher concentrations of diarrheagenic toxins. Consequently, this led to the conclusion that *D. acuta* was by far the most potent toxin producer in the Sognefjord.

6.5 Novel DSP Toxins

The toxins responsible for diarrhetic shellfish poisoning (DSP) were first discovered in the aftermath of cases of human intoxication caused from shellfish consumption in Japan (Murata *et al.,* 1982). Okadaic acid (OA) and analogues such as dinophysistoxin-1 (DTX1) are commonly found in shellfish, particularly from Japan and northern Europe. Since these common analogues have been known and characterized for almost two decades (Yasumoto *et al.,* 1985), they should no longer be considered as "novel or emerging toxins". Other analogues, such as DTX2, first identified from Irish mussels (Hu *et al.,* 1992) appear to have a more restricted geographical distribution in shellfish. In all cases, the DSP toxins have been associated with various species of dinoflagellates from the genera *Dinophysis* and *Prorocentrum*.

The DSP toxins are long chain polyether compounds containing trans-fused or spiro-linked rings with an α-hydroxy carboxyl function, differing only in the number of methyl groups at C31 and C35 (reviewed by Quilliam, in press). A number of acylated DSP toxins may be formed via metabolism in shellfish and these are not *de novo* products of toxin-producing plankton. For example, DTX3-type compounds contain saturated or unsaturated C_{14}-C_{18} fatty acyl groups attached through the 7-hydroxy function of DTX1 (Yasumoto *et al.,* 1985). Acylated forms of other parent toxins, OA, DTX1 or DTX2, have also been found in shellfish in recent years.

In addition to novel DSP toxins formed as metabolic products in shellfish, an array of emerging DSP toxins found primarily in species of benthic dinoflagellates, such as *Prorocentrum lima* and *P. maculosum* has been elucidated. In these novel analogues of OA, the carboxyl group is conjugated to several different unsaturated C_7 to C_9 diols to form allylic "diol esters" (reviewed by Quilliam, in press). *Prorocentrum* species also produce some water-soluble derivatives of OA, in which the diol esters are further conjugated to a polar side chain. In DTX4, the OA moiety is coupled via a C_9 diol group to a C_{14} aliphatic chain with three hydroxyl and three sulphate groups (Hu *et al.,* 1995b), whereas in dinophysistoxin-5 (DTX5a and DTX5b) (Hu *et al.,* 1995c) an amide function is present in the polar side chain. Dinophysistoxin-4 and -5 have not been observed in shellfish, probably because of their lability to esterases in the plankton or shellfish tissues (M.A. Quilliam, unpubl. data).

A wide variety of DTX4 and DTX5 analogues have been detected in various *Prorocentrum* species (Quilliam *et al.,* 1996), based on either OA or DTX1, coupled via different diol linkages to aliphatic chains modified with varying numbers of sulphates and alcohol functions. Although these compounds do not appear to be active as phosphatase inhibitors, they can be hydrolysed chemically or enzymatically to yield the active parent DSP toxins, and thus pose a potential human health risk. The latter is an important point, since Quilliam and co-workers (1996) showed that both ester linkages in DTX4 are easily hydrolysed by an esterase that is present in the plankton and that is released during sample preparation. Conversion of DTX4 to the diol ester proceeds very rapidly (in less than a minute at room temperature), whereas the conversion of diol ester to OA proceeds much more slowly (over several hours). In any case, hydrolysis of these emerging toxins would yield toxic derivatives that would be detectable by conventional chemical analysis (HPLC-FD) and/or DSP mouse bioassay.

6.6 Pectenotoxins

Pectenotoxins (PTXs) were first isolated from the Japanese scallop, *Patinopecten yessoensis* (Yasumoto *et al.,* 1985), as a component of the "DSP toxin group". The PTXs comprise several cyclic polyether macrolides (see Fig. 1). The main toxin, PTX2, is produced by *Dinophysis* species, such as *D. fortii*. As cited in Quilliam (in press), when accumulated in shellfish, the methyl group at C43 is oxidised to the alcohol (PTX1), aldehyde (PTX3), and carboxylate (PTX6) forms. The spiroketal ring system in rings A and B can also undergo rearrangement and/or epimerisation under acidic conditions to produce PTX4 and PTX7 through PTX9. The lactone ring in PTX2 may be opened to yield pectenotoxin seco-acid (PTX2sa), epimerisation of which yields 7-*epi*-PTX2sa. This transformation has been shown to occur enzymatically in shellfish (Suzuki *et al.,* 2001) and even in plankton that has been taken through a freeze/thaw cycle (M.A. Quilliam, unpubl. data).

Toxicological and epidemiological data on pectentoxins (PTXs) are scarce, and the action mechanism of these toxins has not yet been fully elucidated (reviewed by Draisci *et al.,* 2000; Fernández *et al.,* in press). By i.p. injection into mice, PTX1 has been shown to cause damage to the liver, but no diarrhoea was observed (Terao *et al.,* 1986). Pectenotoxin-2 is known to elicit extremely potent cytotoxic activities (at nM to pM concentrations) against numerous human cancer cell lines (Jung *et al.,* 1995), and by oral administration into mice, can cause diarrhoea and severe injuries to the liver and intestine, with oral toxicity very similar to i.p. toxicity (230 μg kg^{-1}) (Ishige *et al.,* 1988). As members of both the OA and PTX groups are produced by *Dinophysis* species, it is very difficult to assess separately the contribution of PTX to human intoxication. Recently, the presence of PTX2 seco-acid in shellfish was correlated with a toxic outbreak in Australia (Quilliam *et al.,* 2000), but the co-occurrence of DTX esters makes this relationship to illness difficult to confirm.

6.7 Broad Spectrum Phycotoxin Surveys

The advent of advanced methods for toxin analysis, particularly sophisticated LC-MS methods for the analysis of multiple toxins from a single injection, and improvements to LC-FD methods, has allowed for broad-spectrum geographical screening of toxins in shellfish and plankton. For example, during a research cruise in May 2000, surveying the east coast of Scotland (reported by B. Luckas, ICES-IOC WG 2002 meeting), samples were analysed for PSP, DSP, ASP, AZP, and PTX toxins. A recent survey (M. Quilliam *et al.,* unpubl. data) of shellfish and plankton samples from diverse locations along the Atlantic coast of Canada (reported by A. Cembella, ICES-IOC WG 2002 meeting), primarily by LC-MS, confirmed the presence of previously well-known and emerging toxins, including those associated with ASP, DSP, PSP, spirolide toxicity and PTX, but the was no evidence of the occurrence of AZA or YTX toxins.

6.8 New and Emerging Toxins in Ireland (2001–2002)

Reported by Joe Silke (Ireland)

The history of the emergence of the azaspiracid (AZA) problem was described in the 2001 WGHABD report. A risk assessment carried out by Irish authorities was summarised. This report recommended an AZA threshold level of 0.1 μg g^{-1} of whole shellfish soft tissue. The Irish shellfish-monitoring programme adopted an interim operational level of 0.2 μg g^{-1}. Chemical testing of all shellfish production areas commenced during spring, 2001. A EU level of 0.16 μg g^{-1} of whole soft tissue was decided upon and has been drafted into new regulations recently ratified by the EU Standing Veterinary Committee.

Azaspiracid continued to be detected in several areas in 2001, at levels necessitating the prohibition of shellfish harvesting in Ireland. Recently, detection of AZA in UK and Norway has been reported and accepted for publication (James *et al.,* 2002, *Toxicon*, in press). The emergence of AZA as a European-wide problem has serious implications for the monitoring programmes in each country, and also places new emphasis on studies to determine the causative organism.

The toxicity of AZA extracted from Irish mussels has been further investigated (Ito *et al.,* 2002). Prolonged periods are required for mice to recover from a single administration of a high (300–450 μg kg^{-1}) dose, and injuries to stomach, small intestine, lung and other organs have been reported. Furthermore, repeated lower AZA doses (1–50 μg kg^{-1}) administered twice a week to observe long term effects on mice caused 20% (n=20) of the test subject to exhibit tumours in the lung. The implications of this study need to be considered by both public health officials and the seafood industry.

The biogenic source of AZA has yet to be published, however, there are strong indications it is associated with *Protoperidinium* spp. A suite of vertical plankton hauls were taken on a research cruise along the west and south coasts of Ireland in July and August, 2001 by the Irish Marine Institute. Early results from the analysis of these plankton hauls indicate that AZA is present at low concentrations at the majority of the sampling sites. The correlation with phytoplankton species has not been completed, but several species of *Protoperidinium* were detected, including *P. crassipes, P. depressum, P. oblongum, P. bipes, P. brevipes* and *P. steinii.*

6.9 New and Emerging Toxins in Norway (2001–2002)

Reported by Einar Dahl (Norway)

Recently improved methods for detection of lipophilic marine algal toxins by LC-MS were implemented at the Norwegian School of Veterinary Science. Using these methods, the number of toxins detected within the DSP and YTX toxin complexes in Norwegian shellfish increased. In 2001, carboxy-YTX and DTX-2 were found by selected ion monitoring (SIM) MS and verified by chromatographic studies with LC-MS and LC-MS/MS. These toxins have not previously been detected in Norway. AZA 1–3, previously only reported in trace amounts, were also found by SIM and verified by chromatographic studies with LC-MS and LC-MS/MS, the latter at the Marine Institute in Dublin. At a few sites, the levels of YTXs and AZAs were above regulation levels and hindered harvesting of shellfish for short periods. During a bloom of *Protoceratium reticulatum* (= *Gonyaulax grindleyi*) along the southern coast of Norway, up to 2.2 X 10^3 cells l^{-1} were recorded and the shellfish accumulated YTX above regulation levels. From analyses of algal cells, picked from net-hauls collected during the bloom, *P. reticulatum* was confirmed as a source organism, containing 16–20 pg YTX $cell^{-1}$.

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7 HISTORICAL DATA

Term of Reference.d: Continue examining the ways of analysing historical data.

Reported by Jennifer L. Martin (Canada) with co-authorship of Fred H. Page and Alex Hanke

A phytoplankton-monitoring programme was initiated in 1987 in the Bay of Fundy, Atlantic Canada. Although in the early years of the programme samples were collected at 17 stations, only four of these stations were sampled consistently until the present. Samples were collected from the surface at all locations except at a site offshore, which was sampled at depths of 10, 25, and 50 m. Samples were preserved in formalin:acetic acid for phytoplankton enumeration and counted using the Űtermohl method. Although a number of variables, such as temperature, salinity, nutrients (nitrate, phosphate, ammonia, and silicate), and chlorophyll *a* have been measured, a preliminary approach has been to look independently at the phytoplankton data.

Phytoplankton results from 1988–2001 have been entered into an ACESS database that includes more than 70,000 records. Data were analysed using PRIMER (Plymouth Routines in Multivariate Ecological Research) software designed for univariate, graphical and multivariate analysis of population data.

Results were initially grouped into three categories: diatoms, dinoflagellates and "other" (including smaller zooplankton, silicoflagellates and ciliates). Multi-dimensional scaling (MDS) analysis indicates spatial differences exist between 12 of the stations monitored at regular intervals throughout 1991. The majority of these stations show various degrees of similarity to the two estuarine stations and the offshore station depending upon location. Phytoplankton populations tend to be relatively low in the winter and late fall with fewer species present, increasing to a maximum throughout the summer. Multi-dimensional scaling (MDS) ordination plots; based on the Bray-Curtis similarity index, show a cyclical and serial pattern in species composition and abundance that corresponds with seasonal changes in community structure.

During the 14-year period *Alexandrium fundyense* concentrations were less than the total numbers of remaining dinoflagellates, except in mid-July during the years 1987, '88, '89, '91, '93, '95 and '98. *Alexandrium fundyense* occurred in samples collected between Julian days 125 and 300 with the highest concentrations generally observed around day 200. A comparison of samples collected offshore at various depths indicated that higher concentrations occurred near the surface.

Cells of the *Pseudo-nitzschia delicatissima* group (including *P. pseudodelicatissima*, *P. delicatissima*) were observed in samples at all locations throughout the year, with higher concentrations occurring between Julian days 165 and 260. Although these cells were observed throughout the water column, higher cell concentrations occurred at 25m. During the years 1988 and 1995, concentrations exceeded 1.0×10^6 cells l^{-1}.

These analyses will be extended to consider inter-annual variability and possible comparisons between regions and data sets.

8 REAL-TIME OBSERVATION WORKSHOP

Term of Reference e: Review progress in the organization of a workshop on real-time observation systems in coastal ecosystems for studies of harmful algal blooms.

Reported by Bengt Karlson (Sweden)

A meeting to plan the workshop was held in Villefranche, France from 4–5 February 2002, with eight participants. During the meeting, a proposal for the European Union programme for "Accompanying measures" was drafted and submitted on 15 February 2002. The *Workshop on Real-Time Coastal Observing Systems For Ecosystem Dynamics And Harmful Algal Blooms* will be held in Villefranche, France, 11–21 June 2003, if funding is provided. Marcel Babin (France), and John Cullen (Canada) will convene the workshop. The Organising Committee includes Jim Aiken (United Kingdom), Allan Cembella (Canada), Hervé Claustre (France), Tommy Dickey (USA), Bengt Karlson (Sweden), Joseph Hun-wei Lee (Hong Kong), Collin Roesler (USA) and Vincent Fournier-Sicre (France).

The workshop will consist of lectures, contributed presentations, posters, tutorials and demonstrations. It is anticipated that 90 people, including 40 invited lecturers and demonstrators, and 50 participants will attend the workshop.

A copy of the detailed announcement that has been prepared for this Workshop is attached as Annex 4.

The Workshop is enthusiastically supported by the WGHABD, which recommends a strong focus on harmful algal blooms be maintained.

A draft resolution requesting ICES endorsement of this Workshop (WKHABWATCH) is in Section 14.3.

9 PROBE TECHNOLOGIES

Term of Reference f: Evaluate progress in the application of molecular probe technologies for a) taxonomic and genetic studies, b) the detection and enumeration of HAB species, and c) the investigation of their physical condition.

Reported by Don Anderson (USA) and Allan Cembella (Canada)

9.1 Background

A common problem in monitoring programs focused on phytoplankton species occurs when the "species of interest" is only a minor component of the planktonic assemblage. Many potentially useful measurements are not feasible because of co-occurrence of numerous organisms of other taxa, as well as detritus. Another constraint arises from difficulties in identifying and distinguishing among morphologically similar species or strains. This is a problem not only for those with limited taxonomic training, but also for skilled taxonomists, since considerable time and effort are required to identify a taxon if the distinguishing characteristics are difficult to discern under the light microscope. For example, certain species of *Alexandrium*, such as *A. tamarense, A. fundyense and A. ostenfeldii* are difficult to distinguish reliably under conventional microscopy, without detailed critical taxonomic observations of individual cells. Such fine levels of discrimination are often not feasible in monitoring programs or studies that generate large numbers of samples for cell enumeration, a situation encountered frequently in studies of HABs.

There is an additional complexity in the fact that cellular- or strain-specific toxicity does not always track identification based upon morphospecific criteria. For example, there are both toxic and non-toxic variants of the dinoflagellate *Alexandrium tamarense.* Among certain strains of the diatom *Pseudo-nitzshia multiseries* with the capacity to produce domoic acid, the induction of toxin production by physiological stress may be required (Bates, 1998). Furthermore, although the toxin profile (relative composition of various analogues) is typically rather constant and presumably genetically fixed within a strain, the cell quota of toxin may vary dramatically in natural populations as a result of multiple extrinsic and intrinsic factors (Wright and Cembella, 1998).

As a result of these problems and constraints, the scientific community has been working towards the development of species- or strain-specific "probes" that can be used to label only the cells of interest so they can then be detected visually, electronically, or chemically. Progress has been rapid and probes of several different types are now available for many of the harmful algae, along with techniques for their application in the rapid and accurate identification, enumeration, and isolation of individual species. Reviews of these methods are available in Anderson (1995), Scholin and Anderson (1998), Anderson *et al.* (2001) and in a new chapter (Scholin *et al.,* in press) in the second edition of the Manual for Harmful Marine Microalgae (Hallegraeff *et al.,* in press.). The latter has been used extensively in the following review, which provides a brief summary of the different probe types (antibodies, oligonucleotides, and lectins) and their applications.

With respect to toxin-specific probes applied to various HAB taxa, most methods are variants of diagnostic assays developed for the detection of the particular toxins in shellfish tissues or other matrices. In a few cases, these assays have been configured with fluorescent or other labels for the detection of toxins within individual cells or at least within a relatively few cells harvested from a mixed population. Compared to molecular probes for the discrimination of HAB taxa, there has been relatively less research (or at least success) in developing toxin-specific probes that can function effectively at the cellular level. For a detailed review of alternative methods for *in vitro* assays for phycotoxins, some of which could be or have been adapted for the detection of phycotoxins in algal cultures and natural plankton assemblages, the relevant chapter (Cembella *et al.,* in press) in the Manual for Harmful Marine Microalgae (Hallegraeff *et al.,* in press) should be consulted. For detailed comprehensive reviews of emerging and developed technologies, including *in vitro* methods, for phycotoxin detection and quantitation, other recent publications (e.g., Towers and Garthwaite, 2001; Van Dolah and Ramsdell, 2001) are also useful.

9.2 Taxon-Specific Probes

9.2.1 Antibodies

Shapiro *et al.* (1989) provide a useful review of the application of immunological techniques to marine phytoplankton identification. The approach involves the use of antibodies that bind specifically to proteins in the cell walls of the algal species of interest. Antibodies are produced by inoculating cells of target species into animals, which then produce antibodies in response to the presence of the intact foreign organism or compounds derived from it. The target molecule against which the antibody is directed (termed an antigen) is typically, but not necessarily, a cell-wall protein. Fortunately, it is not necessary to purify specific proteins in order to produce antibodies.

Most immunological assay methods for cell identification use indirect immunofluorescence for visualization or detection of the label (e.g., Anderson *et al.,* 1989). Visual detection of the labelling is possible using an epifluorescence microscope. Alternatively, samples can be processed using a flow cytometer or other instrument that can detect and quantify fluorescence. Assays can also be conducted using fluorescent or colourimetric detection.

The list of the harmful algal species for which high-specificity polyclonal and monoclonal antibodies (PAbs and MAbs) have been developed is given in Table 2 (from Scholin *et al.,* in press).

Table 2. Established antibodies for identifying HAB species (Vrieling and Anderson, 1996; Scholin and Anderson, 1998; Peperzak *et al.,* 2000).

Species	**Type of antibody**[1]
Bacillariophyceae	
Pseudo-nitzschia pungens	PAb
P. multiseries	PAb, MAb
Cyanophyceae	
Microcystis spp.	PAb
Chrysophyceae	
Aureococcus anophagefferens	PAb
Pelagophyceae	
Aureoumbra lagunensis	PAb
Raphidophyceae	
Chattonella antiqua	MAb
Chattonella marina	MAb
Dinophyceae	
Alexandrium catenella	MAb
A. tamarense	PAb, MAb
A. fundyense	MAb
A.lusitanicum	PAb
A. minutum	PAb
Gymnodinium catenatum	PAb
Gymnodium nagaskiense	MAbs[2]
Gyrodinium aureolum	MAb
Prorocentrum lima	PAb
P. minimum	PAb

[1] MAb = monoclonal antibody; PAb = polyclonal antibody
[2]Two different monoclonals were established; see Nagasaki *et al.* (1991) and Vrieling *et al.* (1994).

Applications of antibody probe technology to field populations are limited to date. Experience with a PAb for the brown-tide organism *Aureococcus anophagefferens*, a MAb for the fish-killing alga *Karenia mikimotoi* and another for *Alexandrium* spp., suggest that immunofluorescence has a major role to play in HAB monitoring and research programs. The antibody for the brown-tide organism has been used for cell enumeration and grazing studies, and to map the geographic distribution of this species over a large region (Anderson *et al.,* 1993). This antibody is now used for routine monitoring of this harmful species. A MAb to the same organism is now available, as well, and it is being used for whole cell assays and in a semi-automated ELISA plate format (D. Caron, *pers. comm*.).

Vrieling and co-workers developed and tested a series of MAbs to *K. mikimotoi* using cultured and natural samples. A direct labelling technique is now used to identify this species at densities of about 1.0 X 10^3 cells L^{-1} in a tube-assay format combined with flow cytometry (Vrieling *et al.,* 1996) and 1.0 X 10^2 cells L^{-1} in a filter assay followed by epifluorescence microscopy (Peperzak *et al.,* 1998). These assays have been used for routine detection of *K. mikimotoi* in Dutch coastal waters since 1996.

Finally, a MAb has been applied in field studies of *Alexandrium tamarense* in the Gulf of Maine, USA (D.M. Anderson, unpub. data; Townsend *et al.,* 2001). This antibody is used in a whole cell format in which samples are labelled and then the species of interest is counted by epifluorescence microscopy. The antibody approach has greatly accelerated the counting of the many field samples that are collected on multiple cruises during the bloom seasons, since the samples can be scanned at low magnification. During these studies, a problem has arisen due to unexpected labelling of *A. ostenfeldii* with the antibody. At certain times, this species co-occurs with *A. fundyense* in the Gulf of Maine, so cell counts of the latter can be inaccurate if antibody labelling is used as the sole identification criterion. As a result, enumeration of both species now requires specification of both cell size and morphological criteria (food vacuoles).

A novel application of antibody technology was reported by Aguilera *et al.* (1996), who used magnetic beads coupled to a MAb to *A. tamarense* to separate the cells of this species from mixed plankton samples after fixation. A more recent development of this method (Aguilera *et al.,* in press) achieved immunomagnetic separation of living cells, allowing several different types of physiological analyses to be conducted on a target species (e.g., rates of primary production and enzymatic activity, cell quota measurements of chlorophyll, protein, etc.). In a direct application of this approach,

A. tamarense/ fundyense cells have been immunomagnetically isolated from Gulf of Maine field samples and assayed for urease activity (Dyhrman and Anderson, unpub. data). This technology thus has great potential for species-specific physiological measurements on HAB species for which cell surface antibodies exist.

In summary, antibody probes have been developed for a number of key HAB species, though more emphasis has been placed on oligonucleotide probe development (see below) in recent years. For some species (e.g., *A. anophagefferens*), antibody probes are the method of choice for cell identification and enumeration, but for others, cross-reactivity problems have limited applications. Finally, the use of magnetic beads with cell surface antibodies offers the possibility of cell separation and thus species-specific physiological measurements. There is thus sufficient benefit and potential for antibodies that development efforts should be continued, in parallel with development of oligonucleotide probe technologies.

9.2.2 Nucelotide probes

In recent years, use of nucleic acid probe technology to detect microorganisms has expanded considerably. This technology is used extensively in the detection of pathogenic bacteria and other microbes, and is now being applied to HAB species. The procedure involves the detection of target nucleic acid sequences by binding (hybridizing) those sequences to a short strand of DNA containing a homologous complementary sequence. Extraordinary sensitivity and specificity are possible with well-designed probes. Many DNA or RNA sequences can be targeted in the organism of interest, including fragments of genes, spacer regions between genes, repeated (non-transcribed) sequences, and transcribed genes.

The first step in probe development is the identification of a unique series of RNA or DNA bases that are only found in that organism. Typically, target genes have sequence domains that are highly conserved among all organisms, and that are thus not useful in discrimination, as well as other domains that are variable to different degrees. It is the latter that are the target areas for probe development. If resolution is sought at the genus, species, or even sub-species levels, the most rapidly evolving, highly variable, domains are targeted, such as those in the intergenic transcribed-spacer (ITS) regions of ribosomal RNA (rRNA). Short, contiguous segments (approximately 20 nucleotides) of nucleic acid sequences are identified and serve as targets for probes. “Oligonucleotide” probes are synthesized and used in a variety of formats to detect the cells of interest.

Oligonucleotide (DNA) probes for identifying HAB species applied in the whole cell format are typically directed against sequences of the small subunit (18S or SSU), large subunit (28S or LSU) and the intergenic transcribed spacers (ITS1, ITS2) of the rRNA cistron (reviewed in Scholin, 1998). Much of this work, especially as it relates to field surveys, has focused on species of *Alexandrium, Pseudo-nitzschia,* and *Pfiesteria* and *Pfiesteria*-like organisms, but *Heterosigma akashiwo*, *Chattonella* and *Fibrocapsa* (J. Tyrell, pers. comm.; Edvardsen and Medlin, unpub. data), and *Dinophysis* spp. (Marín *et al.*2001a; 2001b; Edvardsen and Medlin, unpub. data; Rehnstam-Holm *et al.*, in press), have also been sequenced and targeted for probe design.

One way to use these probes is through fluorescent *in situ* hybridization using intact cells that are either immobilized on a microscope slide or suspended in solution. In this “whole cell” format, the probe enters the cell and binds to target sequences, excess probe is washed out, and the complex is detected by fluorescence or radioactivity. As with antibodies, oligonucleotide probes can be labelled with a variety of fluorescent dyes.

Some new assays immobilize extracted DNA on a solid surface such as a nitrocellulose or nylon membrane to which the probe is added and allowed to hybridise. Excess unbound probe is washed off, and the hybrid (target + probe) sequence is detected using radioactivity, fluorescence, chemiluminescence, or colourimetric methods. There are modifications of this procedure, such as the sandwich hybridisation assay (SHA) in which two probes are used - one to capture the target DNA and bind it to a solid surface, and the other to permit detection. For HAB species, the SHA technique involves collection of a sample onto a filter, after which a lysis solution and heat are used to break cells and liberate nucleic acids. The resulting cell lysate is then dispensed to a pre-packaged 96-well test plate and processed automatically in a relatively simple benchtop system (Scholin *et al.,* 1997). One hybridisation reaction captures the target nucleic acid sequences (DNA or RNA) from the crude lysate using an oligonucleotide tethered to a solid support, and a second reaction binds a signal probe to a different sequence on the target nucleic acid. Visualization of the probe "sandwich" can be enzymatic, yielding colourimetric- or chemiluminescent-products that provide a measure of the abundance of target species in the original sample (e.g., Scholin *et al.,* 1999). Sandwich hybridization offers a potentially faster mode of sample processing than whole cell assays, especially when large numbers of samples must be processed rapidly. Simultaneous detection of multiple species in a single sample is also possible. The assay can be performed in the laboratory as well as aboard ships.

Sandwich hybridisation assays have been devised for *Alexandrium tamarense/catenella/fundyense, Pseudo-nitzschia australis*, *P. multiseries*, *P. pseudodelicatissima*, *P. pungens*, *Heterosigma akashiwo*, *Fibrocapsa japonica*, *Chattonella antiqua/subsalsa*, and a cryptoperdiniopsoid species (from Florida, USA) not yet formally described (Scholin *et al.,* in press). At the time of this writing, prototype SHA kits are available from the Saigene Corporation (Seattle, WA, US), but the kits are still undergoing an active phase of development, testing and refinement.

Detection methods for HAB species that employ the polymerase chain reaction (PCR) have also been developed. Once a potential signature sequence is identified, a pair of oligonucleotide primers (forward and reverse) is designed to bind to unique sequences within or bordering that target. The PCR reaction is run in a thermalcycler that regulates temperature during the reaction process. Reaction products are generally visualized by agarose gel electrophoresis followed by staining with ethidium bromide, SYBR green, or some other nucleic acid stain.

Recently, Bowers *et al.* (2000) described real-time PCR assays for *Pfiesteria* sp. In this assay, an oligonucleotide probe with both a fluorophore and a quencher molecule (Taqman™) are used in addition to oligonucleotide primers, and an instrument capable of excitation and detection of fluorescent signals. Fluorescence is related to the number of amplicons (= free fluorescent molecules in solution) and thus to the number of target molecules in the initial reaction mixture.

Yet another PCR-based method is the heteroduplex mobility assay (HMA) (Uribe *et al.,* 1999; Oldach *et al.,* 2000). These are particularly valuable for identifying unknown cultures or for determining the purity or clonality of a culture. Their use in evaluation of field samples can be problematic, however, since a field sample containing multiple species of the taxon of interest will generate multiple bands that can rapidly become impossible to analyse.

With respect to applications on HABs in natural waters, the SHA, as well as whole cell assays using rRNA probes, have been used in field trials in several areas of the world, including both the east and west coasts of the U.S. (C. Scholin and D.M. Anderson, unpub. data), off the coast of Scotland (John *et al.,* 2002), and in several countries where *Pseudo-nitzschia* species cause ASP toxicity (C. Scholin, unpub data). The most extensive field applications of PCR-based molecular probe technologies to HAB species are probably in the monitoring for *Pfiesteria piscicida* and other *Pfiesteria*-like species in the southeastern U.S. Heteroduplex mobility assays, as well as real-time PCR, have been used for several years in numerous state monitoring programs, while whole cell rRNA probing has been used by Rublee *et al.* (1999) in field studies of *Pfiesteria* species. These molecular techniques have proven invaluable in detecting and enumerating *Pfiesteria*-like species, which are otherwise difficult to distinguish from each other and from co-occurring gymnodinioid forms.

One problem area has arisen with the application of both whole cell and SHA technologies to field populations – namely the agreement between cell counts made with different methods. For example, *A. fundyense* counts using an rRNA probe in the whole-cell format agreed to a variable extent with SHA analyses of the same samples from the Gulf of Maine (D. Anderson, unpub. data). At some stations and at some depths, agreement was excellent between the two methods, but for others, the SHA counts were 2 to 20X higher than the manual counts. It is possible that this discrepancy is due to grazing, perhaps resulting in the incorporation of *A. fundyense* cells and/or rRNA in fecal pellets or other detritus that was detected by the SHA, but not by the whole-cell method. Laboratory experiments, however, have not supported this hypothesis, so the reason for the discrepancy remains unknown.

In a similar manner, Allan Cembella reported to the ICES-IOC WG meeting (2002) on studies of *Alexandrium* populations off the coast of Scotland in which bright-field microscope counts of Utermohl samples were consistently higher than whole-cell counts using species-specific oligonucleotide probes (John *et al.,* 2002). Here again, the discrepancies are significant – an order of magnitude or more. In this case, the differences are between probe-based, whole-cell counts and standard microscope counts, whereas in the Gulf of Maine data cited above, the differences were between the whole-cell probe approach and the SHA. Clearly more work is needed before probe-based cell counts can be accepted as an alternative to more traditional approaches. The differences thus far are significant and raise important questions about what actually should be counted in research and monitoring programs when attempting to enumerate a particular HAB species.

9.2.3 Lectins

Lectins are non-enzymatic proteins (commonly glycoproteins) that bind non-covalently to specific sugar residues at cell surfaces. Potential binding sites associated with microalgae include cell surface glycoproteins, polysaccharides, and chitin. Fluorescently labelled lectins with a range of different binding specificities have been used to differentiate between algal species and even between clones of the same species. For example, they have been used to discriminate between Spanish strains of the toxic *G. catenatum* and morphologically similar, but non-toxic, *Gymnodinium* species (*G. impudicum*; Costas and Rodas, 1994). Likewise, in Korea, lectins are used as a discriminatory tool for the fish-killing alga, *Cochlodinium polykrikoides* (Cho *et al.,* 1998). Rhodes *et al.* (1995) used lectins to differentiate between

morphologically similar *Karenia* species, and demonstrated that *K. mikimotoi* from Waimangu, New Zealand differed from a Japanese strain of the same morphospecies. The New Zealand form of *K. mikimotoi* also differed from a Korean isolate on the basis of lectin binding. The use of lectins for the differentiation of toxic and non-toxic *Pseudo-nitzschia* species has also been explored and is promising for a given species in a particular geographic region. Using a suite of lectins, Rhodes *et al.* (1998) were able to discriminate between six of seven *Pseudo-nitzschia* species.

One limitation is that several different lectins must be used to identify each species. This is possible if a species is to be identified in a small number of samples, but precludes the use of this method in monitoring programs where every sample needs to be probed.

In summary, results of lectin binding studies clearly demonstrate that binding patterns can differ for "strains" of the same (morphologically defined) species, and that despite some limitations, lectin probes can facilitate laboratory and field studies of HAB species.

9.2.4 Probes as biochemical indicators

Although there has been much speculation about the role nutrient availability may play in the apparent increase of HAB phenomena, our knowledge of *in situ* nutritional physiology is still limited for harmful species. To fully understand why blooms occur it is critical to study nutritional physiology in field populations. However, identifying the *in situ* physiology of harmful phytoplankton species in a manner independent from the rest of the phytoplankton community is difficult. One approach to this challenge is to develop fluorescent, single-cell assays for proteins or enzymes that are nutrient-regulated. This is analogous to the widespread use of molecular probes for enumerating harmful species, but in this case, the targets are functional molecules involved in nutrient metabolism and regulated by cellular nutritional status. Cell-specific diagnostics of nutritional physiology represent a powerful tool to link resource availability to the dynamics of a single HAB species throughout a bloom event.

The probes to be used in such efforts can be antibodies to physiologically important proteins or enzymes, or they can be specially designed chemical compounds that serve as indicators. Examples of the former are antibodies targeting flavodoxin, an indicator of iron nutrition (La Roche *et al.,* 1993) and nitrogenase (Currin *et al.,* 1990), a key enzyme in nitrogen fixation. An example of the use of commercially available chemical compounds as a nutritional indicator is the ELF substrate produced by Molecular Probes, Inc., which produces a fluorescent precipitate when it is cleaved by alkaline phosphatase. González-Gil *et al.* (1999) applied this compound to phytoplankton cells and demonstrated its use as an indicator for phosphorus limitation. Dyhrman and Palenik (1999) further characterized the use of this indicator compound.

9.3 Toxin-Specific Probes

9.3.1 Antibodies

In theory, if not in practice, immunological methods for the detection of phycotoxins have several advantages over chemical analytical methods for the detection of particular toxins in phytoplankton in broad-scale screening programs. The sensitivity of immunodiagnostic assays is typically orders of magnitude greater than the corresponding mouse bioassay or chromatographic method with fluorescence or mass-spectrometric detection. Immunoassays made be configured with picogram detection limits and this approach is very amenable to automation (multi-channel manifolds and micro-plate readers) with little concern for complicated sample clean up and preparation. In the last few decades, there have been several concerted attempts to produce reliable immunodiagnostic test kits for various phycotoxins. Many of these efforts have been hampered by the lack of purified toxins for conjugation and difficulties in producing stable immunogens from relatively low molecular weight toxins (e.g., saxitoxin [STX], domoic acid [DA]). Since toxin conjugates for immunization are typically prepared from only a single, readily available derivative, whereas toxigenic phytoplankton usually contain a suite of chemically related derivatives, cross-reactivity is important in the development of immunological methods.

Antibody methods of toxin detection are "structural assays" that are dependent upon the conformational interaction of the analyte (toxin) with a molecular recognition factor, such as the epitopic binding sites. Thus, cross-reactivity in immunoassays is limited to components with compatible epitopic sites and may not reflect relative biological activity or specific toxicity. Such assays yield only an integrated quantitative value representing a group of toxins, whereas the components may vary widely in specific toxicity. The lack of broad-spectrum cross-reactivity for toxic, naturally occurring analogues has been a major drawback to the use of quantitative immunoassays for screening phycotoxins in naturally contaminated samples; however, this is being overcome by second generation antibodies, where more care is taken in designing the antibody 'receptor site'.

A wide array of different assay configurations may be used for immunodiagnostic tests - these include direct- and indirect-coupling, competitive interaction, and "sandwich" assays, although the low molecular weight of many of the phycotoxins limits the use of “sandwich” assays, due to steric hindrance of simultaneous binding of such toxins by two antibodies. Detection systems for immunoassays commonly make use of a radio-label (RIA), a coupled enzyme reaction (EIA), or a fluorescent marker (FIA), but other detection modes (e.g., chemiluminescence) may also be employed. A protocol for screening shellfish tissues by enzyme-linked immunosorbent assay (ELISA) has recently been advanced by AgResearch, New Zealand, and uses a battery of antibodies with sufficient sensitivity and selectivity to detect all known phycotoxin classes at levels below the regulatory maximum permitted limits (MPLs) (Garthwaite *et al.,* 2001).

An absorption-inhibition ELISA (**SAXITOXIN TEST**^{R}, Institut Armand-Frappier, Laval, Canada) was the first practical attempt to configure a rapid diagnostic immunoassay kit for PSP toxins. In this assay, STX is immobilized on polystyrene batons to competitively bind free STX-antibody from a toxic sample-antibody incubation mixture, and thus the colourimetric signal intensity of the assay varies inversely with STX concentration. The polyclonal antibody by STX used in the **SAXITOXIN TEST**^{R} kit exhibits relatively broad antigen specificity and cross-reacts well with at least two gonyautoxins (GTX2 and GTX3), but there is weak detection of the low potency N-sulphocarbamoyl toxins (Cembella *et al.,* 1990). The polyclonal antibody was used to assay bulk PSP toxins in phytoplankton cultures and in natural phytoplankton assemblages (Cembella and Lamoureux, 1993). Unfortunately, detailed collaborative studies were not completed and the kit is no longer commercially produced.

A direct EIA prepared from a polyclonal anti-STX antibody, configured as a microtitre plate ELISA and as a test strip assay, by conjugation of STX to horseradish peroxidase, showed high sensitivity (3 to 4 μg kg^{-1} tissue) for the detection of STX in shellfish (Usleber *et al.,* 1991). A modified assay for qualitative screening employs a membrane filter in a competitive enzyme-linked immunofiltration assay (ELIFA), a simple, rapid assay that can be performed outside of a well-equipped laboratory (Usleber *et al.,* 1995). The cross-reactivity to NEO is poor, but STX, GTX2/GTX3, and decarbamoyl (dc-) STX are detectible below 800 μg kg^{-1} shellfish tissue. A related ELISA method for the detection of STX in shellfish is commercially available as a test kit (**RIDASCREEN**^{R}, R-Biopharm GmBH, Darmstadt, Germany. This assay is frequently used for toxin assays in shellfish but has not been evaluated with respect to performance with phyotplankton extracts or intact cells.

The recently developed **MIST Alert™** (Jellett Biotek, Dartmouth, Canada) has been shown to be highly effective for the detection of PSP toxins in shellfish (Laycock *et al.,* 2002) and plankton matrices (Silva *et al.,* 2001; 2002). This lateral flow immuno-chromatographic (LFI) assay is available on a platform similar to that of a common home pregnancy test kit and permits screening for PSP toxins in <20 minutes. The polyclonal antibodies have been well characterised for their cross-reactivity and limit of detection for multiple PSP toxins standards (CRMP, IMB, National Research Council, Halifax, Canada). All STX analogues commonly found in shellfish, including the N-sulfo-carbamoyl derivatives, are detected, albeit at somewhat reduced sensitivity for the N-1-OH toxins (Laycock *et al.,* 2002).

Immunoassays to a wide variety of phycotoxins have been produced (reviewed in Cembella *et al.,* in press), including those for the toxins associated with DSP (e.g., **DSP-Check**^{TM}), ASP, NSP, and ciguatera fish poisoning (e.g., **Cigua-Check**^{TM}). In all cases, these assays are targeted to the detection of these respective toxins in fish or shellfish tissue extracts and not as probes for the toxins in plankton cells.

Antibodies produced by AgResearch (Hamilton, New Zealand) have been well-characterized by ELISA (Garthwaite *et al.,* 1998) and are now incorporated into the ASP toxin assay known as the **MIST Alert™ for ASP** (Jellett Biotek Ltd., Dartmouth, Canada), an immunochromatographic assay based on the same platform as previously described for PSP toxins. This test has also been recently applied with success to the detection of ASP toxins in samples from toxigenic cultures of *Pseudo-nitzschia multiseries* and natural plankton assemblages containing toxic diatoms (A. Cembella *et al.,* unpubl. data). The nominal detection limit is 2 to 10 ng on the test strip.

The use of toxin-specific antibodies as probes for toxins in individual cells has generally been much more limited in application than for related antibodies used for the detection of these toxins in plankton extracts. This is largely due to the difficulties in permeabilizing the cells to allow the penetration of toxin-specific antibodies for contact with the antigens, most of which are produced as endotoxins. Nevertheless, Anderson and Cheng (l988) achieved some success in the intracellular localization of saxitoxins in *Alexandrium* cells. After determining the cross-reactivity of an anti-okadaic acid antibody to dinophysistoxin-4 (DTX-4), dinophysistoxin-5 (DTX-5), and an okadaic acid diol ester (Lawrence *et al.,* 1998), Lawrence and Cembella (1999) demonstrated the utility of this antibody in the localization of DSP toxin analogues using both immuno-gold labelling under TEM and fluorescently labelled antibody under epifluorescence microscopy. In all cases to date, immunochemical methods have been found to be far too complex and time-consuming for routine cell analysis.

9.3.2 Functional probes

In contrast to structural assays that depend upon a molecular recognition factor that may or may not be correlated with specific toxity, functional assays are based upon the biochemical action of the toxin (e.g., binding to the ion channels of neuroreceptors). Quantitation therefore tends to correlate well with the specific toxicity of the analyte, in spite of differences from whole animal responses that exist due to variation in mechanisms of toxin uptake and conversion in the body. For matrices that contain several toxic components with a similar mode of biological activity, but which vary in specific potency, such assays should yield an accurate estimate of net toxicity.

Among these functional assay methods (reviewed by Cembella *et al.,* in press) are cell culture (cytotoxicity) assays, neuroreceptor assays, and enzymatic activity tests. Like the antibody methods, these techniques are less controversial and more practical alternatives to bioassays using live mammals. Such assays can be performed using simple extraction procedures as they have greater specificity than whole animal assays and do not require the removal of agents such as heavy metals, which can contribute to false positive responses.

Very low detection limits ($<10^{-12}$ M) may be attained with functional assays for phycotoxins and the methods are reasonably easy to automate for multiple parallel analyses. As for the antibody methods, most functional techniques for toxins in phytoplankton are merely variants of the same assay as developed for use with shellfish or human tissue matrices, and are applied only to the bulk assay of extracted toxins. Non-specific binding is one of the problems associated with functional assays, and its importance cannot be overemphasized. Non-specific binding (e.g., to a neuroreceptor) can be defined as extraneous interaction with the ligand (i.e., toxin) resulting from the presence of bindable non-target components in the sample matrix. This spurious binding of components (fatty acids, proteins, etc.) is unrelated to the analytes of interest (toxins), but, fortunately, tends to be somewhat less in phyoplankton extracts than in shellfish tissue matrices.

Functional *in vitro* enzymatic assays for phycotoxin detection are comparatively rare, but the specific inhibition of protein phosphatase Type 1 (PP1) and Type 2A (PP2A) by certain DSP toxin analogues (OA and DTX1) has been exploited in the development of a phosphatase radioassay using ^{32}P phosphorylase. This assay has been applied to assay naturally-contaminated mussel tissue, extracts of cultured *Prorocentrum lima*, and net tow material from natural phytoplankton assemblages. The same enzyme inhibition assay is also useful for the detection of microcystins, a class of phycotoxins produced by certain cyanobacteria, and other toxins capable of inhibiting PP1. A useful version of this PPase assay, based on colourimetric detection, has been applied for the assay of DSP toxins in shellfish and plankton (Tubaro *et al.,* 1996), and further refinements have been recently incorporated. A fluorescence-detection version of this assay has also been developed and successfully used for the detection of DSP toxins (Vieytes *et al.,* 1997). Although the fluorimetric assay offers better sensitivity, and may be preferred in direct comparisons with the colourimetric version, it requires a fluorescence plate reader. The enzyme-inhibition assay for DSP toxins are applied to bulk extracts of plankton or other tissues and no cell-specific probe variation is currently available.

Receptor assays were initially developed to investigate the properties of ion conducting channels, and to characterize the interaction of various ligands with their channel receptors. There now exist receptor assays for many of the known phycotoxins including those responsible for PSP, ASP, NSP and CFP, all of which exert their first order toxic effects by binding to a certain class of biological receptors. This highly specific interaction with naturally occurring receptors is the basis of the receptor assay approach to phycotoxin detection (reviewed in Cembella *et al.,* in press). The affinity of a toxin for its receptor is usually directly proportional to its toxic potency *in vivo* (with some caveats), therefore for a mixture of toxic congeners, a receptor-based assay will normally yield a response representative of the integrated potencies of those toxins present.

A radio-receptor binding assay for saxitoxin (STX) has been refined (Charleston Laboratory, U.S. National Ocean Service) to simplify the protocol and enhance the overall efficiency of the assay (Doucette *et al.,* 1997). Effective use of the assay in both laboratory and field studies of toxic dinoflagellates, as well as in the detection of PSP toxins in human fluids, has also been demonstrated (Powell and Doucette, 1999). Similar radio-receptor binding assays are now available for domoic acid, tetrodotoxin and ciguatoxins and brevetoxins (see protocols in Cembella *et al.,* in press). A critical weakness of the radio-receptor approach to phycotoxin detection is the requirement for the manipulation of radioisotopes, many of which are rare and not always readily available. None of these radio-receptor assays has been configured as a probe of these respective toxins in intact or individual cells.

The ability of the novel transferring protein molecule known as saxiphilin to bind STX with an affinity in the low nM range has been exploited in the development of an alternative assay. Recently, Llwellyn and co-workers incorporated saxiphilin into a radio-receptor binding assay (Negri and Llewellyn, 1998) and ultimately transferred the method into a high throughput, micoplate format. A comparison of the saxiphilin-based receptor assay with HPLC analysis of extracts from various marine species showed a high degree of correlation when the latter results were expressed as STXeq

(Llewellyn *et al.,* 1998). The saxiphilin-based receptor assay shows good promise as a robust technique for detecting PSP toxins in a wide range of sample matrices, especially when discrimination from TTX is required.

A variety of cytotoxicity assays have been developed for the major phycotoxins and they show promise as a rapid screening technique. For example, a tissue culture technique using an established mouse neuroblastoma cell line (Neuro-2A; ATCC, CCL131) has been developed for the assay of Na^+-channel blocking toxins, and has been refined into a laboratory assay kit (**MIST^{TM};** Jellett Biotek, Ltd., Dartmouth, Canada) with the use a scanning spectrophotometer (Jellett *et al.,* 1995). This kit has performed acceptably in collaborative trials and correlates well with the mouse bioassay, but the cell lines are not robust enough to tolerate shipping under extreme conditions, and the propagation and maintenance of cells requires certain expertise and culture equipment. The method works well on dinoflagellete extracts (A. Cembella *et al.,* unpublished data), but the assay cannot be readily re-configured as a probe for toxins in individual cells. Because the cytotoxicity assay for STX analogues depends on the counter-action of Na+-channel blocking activity with various alkaloids, this general approach can be exploited for the assay of other ion-channel toxins, including tetrodotoxin, brevetoxins and ciguatoxins.

Another recent modification to cell-based assays has led to the development of reporter gene assays for phycotoxin detection (Fairey *et al.,* 1997). These assays employ cell lines such as Neuro-2A and pituitary GH4C1 (ATCC, CCL-82.2) that are stably transfected with a reporter gene construct comprised of the *c-fo*s regulatory region linked to the firefly luciferase gene. The *c-fos* early response gene is activated by changes in ion fluxes, such as those caused by certain phycotoxins. Induction of the reporter gene by a toxin yields a luciferase-catalised light emission, which is read on a microplate luminometer. Selection of a cell line for detection of a given toxin is based on its expression of certain ion channel classes (i.e., Na^+, Ca^{++}). While this group of assays has yet to be evaluated for routine monitoring applications, it has proven useful in beginning to characterise the pharmacologic mode of action for bioactive fractions obtained from certain harmful algae (e.g., *Pfiesteria*) (Fairey *et al.,* 1999).

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10 NEW FINDINGS

Term of Reference g: Report and discuss new findings.

10.1 New Harmful Algal Findings In Norway (2001–2002)

Reported by Einar Dahl (Norway)

From the beginning of March to early April, a large bloom of *Chattonella* spp. occurred in the northeastern part of the Skagerrak and along the southern coast of Norway. In total, about 1100 tonnes of Atlantic salmon in pens at three locations were killed. No deleterious effects on natural fauna or flora as a result of the bloom were observed, and a comprehensive screening for potential toxins were negative in both algal samples, and in shellfish and fish exposed to the bloom. The *Chattonella*-bloom followed directly after the spring bloom of diatoms and occupied the surface layer above the pycnocline. At the time the bloom occurred, water temperature ranged from 1–3°C and salinity from 22–28 psu. Although nitrate and phosphate levels were depleted, a significant amount of silicate remained. In the early stage of the bloom, large and elongated cells dominated (up to 2.0 X 10^6 cells l^{-1}), but as the bloom progressed, much smaller cells became more common. Another raphidophyte, *Heterosigma akashiwo,* was also present in significant numbers. A concentration of 1.3 X 10^7 cells l^{-1} (including both raphidophyte species) was recorded during the bloom.

Strains of the genus *Pfiesteria* have recently been isolated from the Oslofjord. *Pfiesteria piscicida* was identified by genetic methods and light microscopy. The related species *P. shumwayae* was attracted from the sediment by the presence of fish and proved to be toxic. The findings indicate a wide distribution of these potentially harmful species, which so far have not been connected with fish kills in Europe.

10.2 Phytoplankton Dynamics in the Bay of Calvi, Western Corsica, France

Reported by Anne Goffart (Belgium)

A long-term phytoplankton study was initiated in 1979 at an oligotrophic coastal site in the Western Mediterranean. This sampling station (42°34'85" N, 08°43'60" E) is situated near the coast, in the northern part of the Bay of Calvi (Western Corsica, France).

The purpose of this study was to establish baseline data on phytoplankton population in relation to water mass characteristics, and to determine patterns and trends in phytoplankton populations. In 2001, data from analysis by light microscopy were added to identify harmful algal species that could potentially cause damage to the aquaculture industry.

Examination of the development of the winter-spring phytoplankton bloom in the Bay of Calvi showed a drastic reduction in phytoplankton biomass and biodiversity over the last two decades. Between 1979 and 1998, the monthly averaged chlorophyll *a* concentrations at 1m decreased by about 80% during February, March and April. Simultaneously, major changes to hydrodynamic conditions included warmer water, an overall decrease in salinity at 10m depth, longer periods of bright sunshine and lower wind stress. The changes in environmental conditions were large enough to reduce nutrient replenishment of the surface layer prior to the usual period of phytoplankton growth. Decreasing Si availability led to Si-limitation, which caused a reduction in diatom abundance. This resulted in the disappearance of the diatom-dominated pulses and in lower phytoplankton biomass and was accompanied by a shift toward non-siliceous phytoplankton (Goffart *et al.,* in press). Other associated changes in benthic assemblages were also observed.

In 2001, no blooms of potentially toxic species were detected between the beginning of January and the end of April. Three small peaks of *Dinophysis* spp. were observed, reaching a maximum concentration of 16 cells l^{-1} by mid-February.

10.3 Reference

Goffart A., Hecq, J.H., and Legendre, L. 2002. Changes in the development of the winter-spring phytoplankton bloom in the Bay of Calvi (Northwestern Mediterranean) over the last two decades: a response to the changing climate? *Mar. Ecol. Prog. Ser.,* in press.

10.4 Temperature Profiling and Hab Intrusions in Southwest Ireland

Reported by Joe Silke (Ireland)

A reasonable understanding of summer water circulation along the Irish Shelf has been gained through fieldwork since the early 1990s, yet a critical question is how offshore populations of toxic dinoflagellates affect bays with inshore aquaculture operations. In an attempt to study inshore advection of these populations by observing cold water intrusions, several temperature sensors were installed at aquaculture sites in Bantry Bay in summer, 2001. To gain insight into processes within the bay, the sensors were placed every 7–8 m throughout the water column to the seabed. The data presented were collected between 15 May and 8 August from the Roancarrig site on the north shore of Bantry Bay and from the Gearhies site on the southern shore.

Initially, the water column was well mixed, but throughout the summer it became more stratified. Water temperature in the surface layers was 14°C, with occasional pulses of cold water measured at depth (16 June 2001) representing a 4°C temperature drop in 12 hours. *Dinophysis* spp. was observed at several locations within the bay following this cold-water intrusion. Toxicity in shellfish in the bay occurred approximately 7 days later, accompanied by closure of many aquaculture sites in the bay. Cold-water intrusions into the Bay also occurred around 5 July and 18 July. Concentrations of *Dinophysis* cells were observed up to 4.5 X 10^3 cells l^{-1} and the area remained closed to shellfish harvesting for a prolonged period.

Examination of the meteorological data from the nearby Valenti observatory suggests a strong link between wind-forcing and temperature fluctuations within Bantry Bay. The predominant wind in this area is typically from the southwest. The data showed that when the wind shifts to an easterly direction, a sudden lowering of temperature occurs at lower depths.

In future, real-time measurements of ocean temperature and wind in Bantry Bay may be useful to help predict algal blooms. This work also demonstrated a useful means of deploying probes on existing aquaculture platforms, such as salmon cages and mussel longlines, to gather crucial environmental data related to harmful algal events around the Irish coast.

11 VARIABLES AND INDICATORS

Term of Reference h: Prepare a summary report listing relevant marine bio-ecological variables and indicators suitable for operational use.

In general, the WG endorsed the idea that relevant monitoring tools must be developed to yield an early warning of bloom events and to establish triggers for the initiation of sampling and analysis. Critical issues to be considered are: 1) number and location of sampling stations; 2) sampling frequency, 3) methods of sample and data recovery, and 4) data processing and archiving. At a basic species and/or toxin level, this may already be partially accomplished with conventional methodologies, but new methods must be readily introduced as they become reliable enough for monitoring. Examples of such relevant bio-ecological variables include real-time "on-line" access to vertical profiles of salinity, temperature, chlorophyll, and light. Future developments may include access to *in situ* information on the occurrence of particular harmful species, by using probes, moored microscopes, and image processing systems, etc., and toxin measurements by *in situ* assays and deployable analytical systems, with automated collection of samples from moorages. For a limited suite of HABs – those that form high biomass blooms at the surface – airborne- and satellite-based systems have already proven to be useful in describing open-ocean and coastal blooms.

12 OTHER REPORTS

Don Anderson (USA) reported on HAB programmes relating to ECOHAB:

1) A companion programme to ECOHAB called 'Oceans and Human Health' has just been established in the United States. This contains a section on HABs, with genetics, toxin biosynthesis, toxicity, etc.

2) **ECOHAB-EUROHAB Collaboration**

   Given the global expansion of HAB-associated impacts, the need for a coordinated effort to understand these problems is essential. Blooms with similar impacts, both toxic and high-biomass related, affect the shores of Europe and the U.S. An agreement has been signed by officials of the U.S. National Science Foundation and the European Union that will establish procedures for collaboration among scientists from these areas. Research on HABs has been selected as one area for immediate activity. A program is thus being developed to facilitate

research on these phenomena by members of the HAB scientific community from both sides of the Atlantic. The first step in this process will be a joint ECOHAB-EUROHAB workshop, the goals of which are to discuss common problems with respect to HABs and to identify potential topics for joint research. The workshop is tentatively scheduled for the autumn of 2002. An important aspect of this planned collaboration will be comparative ecosystem studies. Such comparisons allow the synthesis of knowledge and data needed to group HABs from similar habitat types and to distinguish the mechanisms controlling their population dynamics. Similar ecosystems should respond in broadly similar ways. Therefore, identification of early warning indicators of system changes within and across ecosystems types will greatly help prediction and possible mitigation of HABs. Comparative ecosystems are a program element of GEOHAB, so this important US-EU activity may become a mechanism for funding such research within that program.

3) **IOC INTER-GOVERNMENTAL PANEL Meeting**

The meeting of the IOC Inter-Governmental Panel on HABs scheduled in May 2002 has been re-scheduled for October 2002.. **The WGHABD recognizes the importance of the IOC Inter-Governmental Panel and encourages and supports governmental managers to attend.**

13 CONCLUDING BUSINESS

An election for new Chair of the WGHABD was held at the Bermuda meeting; Jennifer Martin (Canada) was the successful candidate. The WGHABD thanked Allan Cembella (Canada) for organising and chairing the 2002 meeting, and recognised with appreciation the service of retiring Chair Kaisa Kononen (Finland). The members of the WGHABD also appreciated the efforts of Eileen Bresnan (Scotland), who generously agreed to act as Rapporteur for the 2002 meeting. Balloting for the venue of the 2003 meeting of the WGHABD was held; the WG proposes to meet in Aberdeen, Scotland in March 2003, to be hosted at the FRS Marine Laboratory.

14 DRAFT RESOLUTIONS

14.1 WGHABD 2003 Meeting with Justifications

Term of Reference	Justification
ToR 1: Compare and assess historical and retrospective data sets on phycotoxins in shellfish, related toxic phytoplankton abundance, and phytoplankton community structure with reference to HAB population dynamics.	Studies show that all phytoplankton populations, including those of HAB species, exhibit large interannual variation in bloom intensities. Analyses of HABs for trends and patterns in combination with total community structure, as well as with reference to physical and chemical parameters, are necessary to advance knowledge on HABs. Likewise, analysis of shellfish toxicity in the context of environmental variability can give important insights into HAB population dynamics. As longer time-series data sets become available, it should be possible to hindcast and forecast general impacts of occurrences, distributions, and amplitudes of HABs. Interpreting the dynamics of HABs requires different analytical approaches to data from long-term studies.
ToR 2: Review the reports and products of the upcoming workshops on molecular probe technology, and the development of technologies of direct use in studies of field populations of HAB species, with special attention to novel approaches that were not considered at the 2001 WG meeting.	Molecular probe technology is evolving very rapidly in the HAB field. The current status was reviewed at the 2002 WG meeting, but this information will soon be out of date, particularly in light of methods to be presented at several international advanced workshops on this topic. In addition, a number of problems have arisen in the application of these probes to field populations, thus additional consideration is required to determine the extent to which these have been resolved.
ToR 3: Evaluate the outcome of the Den Haag Workshop: ”Contrasting approaches to understanding eutrophication effects on phytoplankton” from a HAB dynamics perspective.	The significance of eutrophication as a driving force for HABs has been under discussion for a long time, and has been within the purview of the ICES-IOC WGHABD since its inception. A revisit of this complicated and complex theme should be done after the Den Haag Workshop (11–13 March 2002). The WG is particularly concerned that the topic of eutrophication be addressed in terms of HAB “species of interest” and not restricted

Term of Reference	Justification
	to general high biomass phytoplankton blooms. Consideration by the WGHABD will contribute to a realistic assessment of the importance of eutrophication in HAB dynamics and foster linkage to the relevant theme within the GEOHAB program.
ToR 4: Review effects of HABs on survival and fecundity of wild fish, and the relationship (if any) to recruitment into populations in the ICES area.	Over the last decade, there have been several hundred fish kills caused by harmful algal events in the ICES area. Although most of the well-studied cases of mass mortalities are known for species in aquaculture, wild fish mortalities have also been recorded. The mechanism of mortality is usually unclear and poorly understood. Wild fish populations have collapsed or at least become severely depleted in the coastal zones of several ICES countries. Exposure to HABs is one of the hypotheses invoked to explain some of these phenomena. In addition to the acute morbidity and mortality effects of exposure of juvenile and adult fish to HABs, exposure to phycotoxins or other noxious substances produced by algae during fish spawning or during early larval stages, might severely damage an entire year-class of fish. The effect of HABs on fecundity and egg survival is controversial, and the paucity of data compilation and interpretation on this subject warrants a review of existing data and suggestions on relevant research.
ToR 5: Prepare a resolution for a workshop on "New and classic techniques for the determination of numerical abundance and biovolume of HAB-species – evaluation of the cost, time-efficiency and intercalibration methods".	Techniques for estimating abundance and biovolume of HAB species are essential to the study of HAB dynamics and for evaluating consequent effects on ecosystems. Classic techniques, such as the Utermöhl microscopic method are time-consuming and require highly skilled personnel for identification. In many cases, taxa cannot be readily identified to the species level. Recently, new techniques, such as antibody-labelling and oligonucleotide probes, have become available. These can be automated and may be able to process samples faster than was possible previously. These techniques are very promising and may revolutionise HAB research, but they must tested thoroughly. Intercalibrations with classical methods are essential. Furthermore, other techniques directed towards producing rapid results for use in operational oceanography and for early warning of HABs for aquaculture must be compared with classic techniques. Deconvolution and other techniques for enhancing images from bright-field, phase-contrast and epifluorescence microscopy are available. Similarly, improved image-analysis methods and neural network approaches show excellent promise. There is a need to evaluate these methods for use with HAB species.
ToR 6: Evaluate the usefulness and feasibility of creating HAEDAT maps directly from the HAEDAT-database.	Evaluation of the current database has highlighted discrepancies between the HAEDAT database and the decadal maps. Problems to solve include: the need to update maps quickly, the poor flexibility in extracting data depending on user requirements, lack of standardisation of inputs and the need to access and maintain the database with respect to GIS software, such as ARCVIEW or MAPINFO.
ToR 7: Review the application of methods for the detection and quantification of phycotoxins in eukaryotic microalgae and cyanobacteria, and related components of pelagic food webs, in coastal marine and brackish waters of the ICES region	Methodologies for the detection, quantification and structural elucidation of phycotoxins have evolved to the point where they have the potential to be used to resolve critical issues regarding the role of these toxins in HAB dynamics and food chain effects. Improvements in sensitivity, resolution, and signal-to-noise ratio of analytical instruments and assay methods for various phycotoxins, and the increasing availability of analytical

Term of Reference	Justification
	standards and reference materials, have made it possible to identify and quantify such phycotoxins in a few phytoplankton cells, in zooplankton and even from seawater. Several workshops and training courses have introduced these techniques, but they have often been considered from the perspective of shellfish as toxin vectors and consequent effects on human health. A review of the use of these techniques, particularly as applied to cultures and natural populations of phytoplankton and zooplankton, in the context of HAB population dynamics is required.
ToR 8: Review the previous submissions to HAEDAT with a view to improving the accuracy of the information and increasing the utility of the database.	Inconsistencies have been identified by the WGHABD in species names, identification of reporting areas, categorisation of harmful events, etc. Modifications to the input data will improve the quality of the data extracted by the end users.
ToR 9: Report on the ECOHAB-EUROHAB Workshop on joint research on HABs.	A joint research programme between the US and the EU on HABs will be of great interest to many members of the WGHABD. Opportunities for collaborative research on HAB dynamics can be considered and planned by WG members if they are fully informed of the status of this important programme.
ToR 10: Prepare a summary report listing relevant marine bio-ecological variables and indicators suitable for operational use	This ToR, which was given to a number of other ICES WGs should be revisited by the WGHABD. Members were not provided with sufficient background information on this task to adequately address the topic at the 2002 WG meeting.

Supporting information

Term of Reference 1: In order to facilitate this ToR, members of the working group are asked to identify existing datasets on HAB plankton and shellfish toxicity within their countries and document parameters measured such as: depth, sampling frequency, HAB species identified and community structure, periodicity, length of time-series, location, units of measurement and sampling method. This information should be accompanied with information on availability of data and individual to contact.

Selected members of the Working Group will present results from the analysis of the historical data from their region.

Term of Reference 2: Certain members of the working group will report on the workshops on "Molecular Probe Technology for the Detection of Harmful Algae" in Galway, Ireland (May 20–24, 2002) and on "Analysis of Single Cells of Marine Phytoplankton (ASCMAP)" in Bremerhaven, Germany (April 15–21, 2002). Caroline Cusack from the Marine Institute in Ireland will be asked to report on both meetings, highlighting important technical developments relating to HAB population dynamics. If reports or summaries of these meetings are available, these will be requested and presented to the members for their review.

Term of Reference 3: A member of the WGHABD will be attending this workshop and the ICES Phytoplankton Ecology Working Group meeting and will report results relevant to HAB dynamics. If the published report is available, the document will be reviewed and be used in conjunction with other information to plan future activities of the WGHABD related to eutrophication effects on HABs. A representative from the Phytoplankton Ecology Working Group (David Mills) will be asked to participate at next WGHABD meeting to discuss eutrophication issues.

Term of Reference 4: Contributions of data on this topic will be solicited from WG members, e.g., the results of relevant experiments on cod larvae conducted in Norway. In addition, a recognised expert in fish pathology and recruitment processes will be invited to give a presentation on this subject and assist in the review and interpretation of existing data and in formulating recommendations on future research.

Term of Reference 6: WG members will identify end-user requirements in their respective countries, with reference to the database and mapping protocols. Representatives from the IOC-Vigo Centre will investigate and report on technical solutions for the automatic production of maps from the HAEDAT database.

Term of Reference 7: Selected WG members with expertise in analytical and assay technologies for phycotoxins will review and report on developments in the application of these methods to emerging and well-characterised toxins in the context of HAB dynamics.

Term of Reference 8: In consultation with the IOC-IEO SCCHA, WG members will identify and detail current and historical problems in the data reporting protocols. Special attention should be paid to specific information that was not requested on the old form, and also to species names (synonyms or misidentifications), location information, introducing the ICES area code or the region name, etc. A guidance document will be prepared at the next WG meeting to ensure uniform modifications across the database.

Term of Reference 9: A member of the WG, Don Anderson (USA) or other designate, will prepare a report from the ECOHAB-EUROHAB workshop, detailing progress on joint research on HABs for consideration at the next WG meeting.

Term of Reference 10: The WG expects that considerable new information relevant to HABs will be yielded from the proposed Workshop on Real-Time Observations. Nevertheless, this information will not be available until after June, 2003, therefore, considerable intersessional work by selected members of the WGHABD will be required prior to the 2003 WG meeting.

14.2 Workshop on Real-Time Coastal Observing Systems, with supporting information

A Workshop on real-time coastal observing systems for ecosystem dynamics and harmful algal blooms [HABWATCH] (Co-Chairs: M. Babin, France and J Cullen, Canada) will be held in Villefranche-sur-Mer, France from 11-21 June 2003 to:

Through plenary lectures, contributed presentations, demonstrations and practical tutorials:

Review real-time and near real-time sensing systems applicable for observation, modelling and prediction of plankton dynamics in coastal waters, including HABs. Present the underlying theory and review the possibilities together with the current issues and limitations. Topics will include:

a) Remote sensing of coastal waters.

b) *In situ* optical measurements, both passive and active.

c) Automated methods for detection of plankton species or toxins.

d) Integrated observation systems combining various kinds of detectors (optical, chemical, hydrodynamical, …), including moorings and autonomous vehicles.

e) Continuous underway sampling systems (e.g., from ferries).

f) Tools for characterising distribution of plankton in relation to physical and chemical properties.

More through the plenary lectures and demonstrations than through the practical tutorials:

g) Elaborate guidelines for the development of strategic and rational use of optical sensors for specific HABs problems

h) Explore approaches for integrating data from various sensing systems to describe ecosystem processes in support of HAB research, monitoring and prediction (e.g., information systems).

i) Review of prognostic models designed to use real-time observations of variables related to coastal ecosystem dynamics and HABs.

j) Introduce and review data assimilation techniques.

Supporting Information

Priority:	ICES should take an active role in developing the implementing plan of the GEOHAB programme. The topic of real time observations is relevant for GEOHAB and also fits well to ICES profile. This also fits in directly with the objectives of the Coastal Ocean Observation Panel of C-GOOS.
Scientific Justification:	The development of a harmful algal bloom is a result of interactions between the physiological characteristics of the species as well as physical and chemical processes in the environment in which it grows. Therefore dynamics of these blooms cannot be studied without the integration of different observation approaches, instrumentation and methodologies. Real time observations form the basis for adaptive sampling in field studies. Data from various sensing systems need to be integrated into models - data assimilation is a crucial step in model development. There is a great deal of interest throughout the world in the installation of coastal ocean observation systems with capabilities for detection and prediction of HABs. However, many of the approaches are unfamiliar to potential users.
Relation to Strategic Plan:	Implementation of the GEOHAB programme is relevant to quantifying anthropogenic impacts on the marine ecosystem (e.g., eutrophication). This workshop will allow progress to be made in the development of predictive models of toxic events, on the basis of GLOBEC implementations.
Resource Requirements:	Conveners and lecturer's time is required. Travelling and accommodation costs are needed for meeting participants. Conference room and facilities are also required during the workshop. Convenient access to coastal waters, with support for deployment of instruments on a mooring and off a pier would be desirable. Technical support would be required for electronic publication.
Participants:	Experts in relevant fields from around the world would be invited to present overview lectures and to hold tutorials/demonstrations. Other participants, end users and students, would apply for positions at the workshop. Criteria for selection would include direct involvement in the planning for or deployment of coastal observation systems.
Secretariat Facilities:	Secretarial support will be provided by a private conference organiser.
Financial:	Travelling support is needed for presenters and participants. Funds will be asked from SCOR and other relevant organizations. Significant funding is expected from the European Commission, IOC and the Office of Naval Research (US).
Linkages To Advisory Committees:	Harmful algal blooms and eutrophication effects are continuing issues in ACME.
Linkages To other Committees or Groups:	Support can be anticipated from Baltic Committee and Marine Habitat Committee. A letter of support has already been given by the Coastal Ocean Observation Panel of GOOS.
Linkages to other Organisations	GEOHAB is sponsored by IOC and SCOR. The Workshop is also relevant to the interests of GLOBEC and IOC-GOOS
Cost Share	ICES:

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ANNEX 2: AGENDA OF THE MEETING

Date	Time	
Thursday 7 March	7:45–8:30	Breakfast
	9:00–9:30	Opening of the meeting Introduction of the meeting participants Practical information Information from ICES Adoption of the agenda
	9:30–10:40	ToR 2: GEOHAB Report of SSC on Implementation Plan Report on Baltic-GEOHAB (B. Karlson) Report on C-GEOHAB meeting (J. Martin) Other Regional Initiatives (e.g., China) Report on Science Plan Theme meetings (e.g., post-LIFEHAB WS)
	10:40–11:00	Coffee break
	11:00–12:30	ToR 3: New and emerging phycotoxins Presentation on Emerging toxins in ICES region (A. Cembella) Presentation on azaspiracids, spirolides, and PSP toxins in North Sea (B. Luckas) Discussion
	12:30–13:30	Lunch
	13:30–14:00	ToR 5: Workshop on real-time observations Report on Villefranche OC meeting (B. Karlson) Discussion
	14:00–15:00	ToR 4: Historical data
	15:00–15:20	Coffee break
	15:20–17:00	ToR 1: Summary of HAEDAT (M. Leon) Discussion about the data reporting
	18:30–19:30	Dinner
Friday 8 March	7:45–8:30	Breakfast
	9:00–10:40	ToR 6: Molecular Probes Presentation (D. Anderson) Presentation (A. Cembella) Discussion
	10:40–11:00	Coffee break
	11:00–12:30	ToR 1: National reports
	12:30–13:30	Lunch
	13.30–15:00	ToR 1 continuing: National reports and decadal maps
	15:00–15:20	Coffee break
	15:20–	Report writing

Date	Time	
	18:30–19:30	Dinner
Saturday 9 March	7:45–8:30	Breakfast
	9:00–10:40	ToR 8: bio-ecological variables and indicators for operational use
	10:40–11:00	Coffee break
	11:00–12:30	ToR 7: New findings
	12:30–13:30	Lunch
	13:30–15:00	ToR 7 continuing: New findings
	15:00–15:20	Coffee break
	15:30–	Report writing
	18:30–19:30	Dinner
Sunday 10 March	7:45–8:30	Breakfast
	9:00–9:30	Election of the new WG Chair
	9:30–10:30	Discussion of ToRs Adoption of the report
	10:30–11:00	Coffee break
	11:00–12:00	ToRs for 2003 Selection of Meeting place for 2003
	12:00	Close of the meeting

ANNEX 3: PROCEEDINGS OF THE WORKSHOP: "LIFEHAB - LIFE HISTORY OF MICROALGAL SPECIES CAUSING HARMFUL BLOOMS", CALVIÁ, MALLORCA (SPAIN), 24–27 OCTOBER 2001

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ANNEX 4: WORKSHOP ON REAL-TIME COASTAL OBSERVING SYSTEMS FOR ECOSYSTEM DYNAMICS AND HARMFUL ALGAL BLOOMS

11-21 June 2003

Observatoire Océanologique and Citadelle of Villefranche-sur-Mer, France

CONVENORS:
Marcel Babin, LOV/CNRS, France

John Cullen, Dalhousie University, Canada

Sponsors:

GEOHAB (IOC/SCOR), C-GOOS (IOC), ICES, …

Content List

1. Introduction

1.1 Context

There is a great deal of interest throughout the world in the installation of ocean observation systems to provide the data and knowledge needed to detect and forecast physical, chemical and biological changes in coastal and open-ocean ecosystems. Recent advances in instrumentation, communications and modelling capabilities have led to the design of prototype real-time observation and prediction systems for coastal ecosystems. Important phenomena in coastal waters include flooding and coastal erosion, oxygen depletion due to eutrophication, and harmful algal blooms (HABs). Algal blooms are episodic and their development is a result of interactions between ecological and physiological characteristics of the species and the physical and chemical processes in its growth environment, so the detection and prediction of HABs require comprehensive capabilities in real-time ocean observation and modelling. However, many of the approaches are unfamiliar to potential users. Optical and chemical sensors are, for instance, increasingly used from various platforms (*in situ* profiler, towed vehicle, mooring, satellite) to derive quantitative and qualitative information on phytoplankton environment (e.g., light field, coloured dissolved organic matter, nutrients), and on phytoplankton itself (spatial distribution, species composition, cell size, optical and photosynthetic properties). Effective use of these sensors does not necessarily require advanced technical training, but it does require knowledge of the underlying theoretical and technical principles, how to properly deploy these instruments, methods for processing data, approaches for interpreting the results within reasonable limits, and how such results can be incorporated into different kinds of predictive models.

We therefore propose to organise a "Workshop on real-time coastal observing systems for ecosystem dynamics and harmful algal blooms" in Europe on 11-21 June 2003 at the Observatoire Océanologique and Citadelle of Villefranche-sur-Mer, France. The idea of this workshop was initially expressed by the Working Group on Harmful Algal Blooms Dynamic of ICES (International Council for the Exploration of the Sea). At this stage, this initiative is endorsed by both GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms), an international programme sponsored by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC from UNESCO), and the Coastal Ocean Observing Panel (COOP) of the Global Ocean Observing System; it is also encouraged by ICES. However, these programs do not have the financial resources to fund such a large workshop.

This workshop is dedicated to an international audience. Our approach will consist of providing the participants with both the theory relevant to understanding the basic principles of real-time observation and modelling tools, and tutorials to allow the use of these tools. Our expectation is that all participants will then have enough skills to *initiate* the deployment and use of real-time observation and modelling systems, and will understand the current capabilities and limits of these tools.

1.2 Objectives

Through plenary lectures, contributed presentations, demonstrations and practical tutorials:

Review real-time and near real-time sensing systems applicable for observation, modelling and prediction of plankton dynamics in coastal waters, including HABs. Present the underlying theory and review the possibilities together with the current issues and limitations. Topics will include:

a) Remote sensing of coastal waters
b) *In situ* optical measurements, both passive and active.
c) Automated methods for detection of plankton species or toxins.
d) Integrated observation systems combining various kinds of detectors (optical, chemical, hydrodynamical, …), including moorings and autonomous vehicles.
e) Continuous underway sampling systems (e.g., from ferries).
f) Tools for characterising distribution of plankton in relation to physical and chemical properties.

More through the plenary lectures and demonstrations than through the practical tutorials:

a) Elaborate guidelines for the development of strategic and rational use of optical sensors for specific HABs problems (**see text box**)Explore approaches for integrating data from various sensing systems to describe ecosystem processes in support of HAB research, monitoring and prediction (e.g., information systems).

c) Review of prognostic models designed to use real-time observations of variables related to coastal ecosystem dynamics and HABs.

d) Introduction to and review of data assimilation techniques.

During recent years, optical sensors (both *in situ* and remote sensing) have been promoted as magic tools for detecting various aspects of phytoplankton dynamics, including HABs. Some claims have been either idealistic or unrealistic, and consequently some have lost belief in such tools. Therefore, our idea is to make clear during this workshop that optical instruments have significant, but highly specific potential for addressing realistic environmental issues in a practical way. Take, for example, ocean colour detected from space. Considering the limited spatial resolution of existing sensors, sensitivity, and influences of land on nearshore elements, ocean colour remote sensing can be used effectively for studies at meso to large scales (for instance). It is also limited by cloud occurrence, detection at surface only and a reoccurrence time of 2 to 3 days. Imagers on airplanes provide much better spatial resolution, and reoccurrence is flexible; however the cost is higher. The remote detection of phytoplankton groups based on optical signature may be possible for a few groups (e.g., the cyanobacterium *Trichodesmium*, coccolithophores, and possibly species with distinct pigmentation) that develop large patches at the surface, but the routine use of remote sensing for observing coastal phytoplankton dynamics has yet to be comprehensively evaluated. For every sensing modality, we could follow the same approach, describing strengths and limitations and discussing possible applications in observation systems. Thereby, the users would leave with a realistic view of what can be actually done with sensors, how they could be included in their observation strategy, and what can be expected from the data.

2 State-of-the-rt

2.1 Operational and near-operational real-time coastal observing systems

During the past two decades, time series measurements of optical and bio-optical properties using moorings and bottom tripods have advanced to the point where they can now be made on virtually the same time scales as their physical oceanographic counterparts (Glen *et al.*, 2000a, b). Early optical measurements (roughly 1980s) were limited to scalar irradiance (photosynthetically available radiation or PAR), beam attenuation coefficient (660 nm), and stimulated and natural fluorescence. Within the past few years, spectral measurements of inherent and apparent optical properties have advanced from 7-9 wavebands to 100 wavebands. These data sets are allowing us to analyse the temporal evolution of partitioned optical properties according to composition by phytoplankton, detritus, and gelbstoff (e.g., Chang and Dickey, 1999; Claustre *et al.*, 2000). The next step is being directed toward separation of phytoplankton by groups such as those associated with harmful algal blooms (see Schofield *et al*., 1999). For instance, it has been shown in the laboratory (Johnsen *et al*., 1994) and in the marine environment (Millie *et al.*, 1995; 1997; Kirkpatrick *et al.*, 2000), that phytoplankton groups may be discriminated based on the spectral shape of their absorption spectrum (but see Garver et al. 1994). Consideration of absorption in the UV may further improve discrimination (Kahru and Mitchell, 1998)

Time series measurements from moorings and tripods have typically been made at specific sites with complementary data sets provided by satellites and ships in order to provide spatial data context. Scientific breakthroughs have been enabled by use of these and other optical systems (e.g., spectral fluorometers, fast repetition rate fluorometers, flow cytometers, etc.). Some of the interesting results involve observations of sediment resuspension in the wakes of hurricanes, optical variability associated with the passages of internal solitary waves, transient and seasonal phytoplankton blooms (e.g., Cullen *et al*., 1997), and passages of sub-mesoscale features and fronts with specific optical and bio-optical characteristics (e.g., Dickey, 2001). Several ocean colour satellite sensors are or will become soon available with improved spectral and spatial capabilities (IOCCG, 2000). Although the limitations in the use of ocean colour data for HABs detection have been acknowledged (Schofield et al., 1999; Cracknell *et al.,* 2001), especially at the level of discrimination between phytoplankton groups, few studies have shown how this technique can complement *in situ* measurements by providing spatial distribution and temporal evolution of blooms (chlorophyll *a* concentration) (e.g., Gower, 1994; Sathyendranath *et al.,* 1997; Lavender and Groom, 2001).

There are a number of real-time coastal observation systems around the world, and there are a few nascent programs which may be classified as coastal networks that include optical and bio-optical measurements as components. For example, one of the more advanced has been conducted as part of the HyCODE program at the Long-term Ecosystem Observatory (LEO-15) site off the coast of New Jersey, USA. During the summers of 2001 and 2002, an array of sampling platforms was used to study optical variability using hyperspectral optical sensors and systems. Platforms used for this work included moorings, autonomous underwater vehicles, aircraft, and satellites. Time scales of variability of minutes to months and spatial scales from meters (and less in the vertical) to about 100 kilometres are

resolved with these collective observational platforms. Resulting data are being used for several studies including: physical-biological-optical interactions, radiative transfer, optical closure, algorithm development for remote sensing, visibility, and data assimilation modelling. Other field efforts utilising sampling networks of this type are also being developed in Europe. Near real time data (so far mainly physical oceanographic data) is shared among the countries in the North Sea area using a system called SNDI (Sea Net Data Interface). This system needs to be extended. Possible platforms for instruments able to monitor harmful algal blooms include buoys (moorings), light houses, bridges, wind mills and ships of opportunity. Single moorings for various short term research projects in physical oceanography are fairly common. Most of the existing observation networks in Europe are aimed at physical oceanographic variables and meteorology. The German network uses stationary structures somewhat similar to, but smaller than, oil rigs. In Danish waters some bridges are used as instrument platforms but also buoys are operational. In Sweden, two sophisticated buoys were deployed in 2001. Satellite transmission of data makes it possible to deploy these systems in offshore waters. The current instrument set up is not ideal for HAB monitoring. Chlorophyll fluorescence is measured at one depth only which limits the use of the data since sub-surface blooms are common. In the Seine estuary three buoys are in operation. Water is pumped through sensors in the moorings and e.g., concentrations of nutrients and chlorophyll are measured. In the Baltic a system for monitoring HAB using ships of opportunity, i.e., passenger ferries, is in operation (Alg@line http://www.fiMrfi). Salinity, temperature and chlorophyll fluorescence is measured continuously in pumped water and up to 24 water samples can be collected at predefined positions during one voyage. Samples and data are collected when ships reach port. In Norway and Sweden similar systems are being built up but data communication is in near real-time. Also a ship of opportunity monitoring system exists in the English Channel. All ship of opportunity monitoring of HABs are limited by the sampling at a single depth since HABs often develop sub surface. A system called the Continuous Plankton Recorder (CPR) has been in operation using merchant ships since the late 1940s. Although the system is of value where long term series exists, it has little use for monitoring HABs since fragile algae are damaged and analysis of the silk collecting the plankton is very time consuming.

In France, the "MAREL" system deployed by IFREMER in the Bay of Seine will soon reach the number of four moorings equipped with sensors for various basic measurements at three depths (temperature, salinity, pH, dissolved oxygen, nutrients, swell, …). Data are acquired every hour and transmitted in real-time to land. The Seawatch system developed by the Norwegian firm Oceanor has been deployed in different parts of the World, especially in European waters (Baltic, North Sea, Norwegian Sea, Mediterranean Sea, Barents Sea, Atlantic Ocean). The Seawatch moorings also provide real-time measurements of various properties of coastal ocean surface. The Italian firm Idronaut has also developed a coastal observing system that allows profiling over the water column with measurements of different water properties. This system includes few optical sensors (fluorometer, transmissiometer). Important components of future optical sampling networks will involve real-time or near real-time data telemetry and data assimilation models allowing predictions and knowledge-based decision-making (Dickey and Chang, 2001).

2.2 Emerging technologies for HABs study/monitoring

Routine monitoring of Harmful Algal Blooms (HAB) is subject to severe methodological constraints due to dependence on conventional counting and identification of specific organisms by optical microscopy. This conventional approach is tedious and time-consuming, requires a high level of taxonomic expertise and can only be applied to discrete samples. In cases of taxa that are difficult to identify, accurate quantification may require access to scanning electron microscopy (SEM) or fluorescence staining techniques, further adding to the delay and complexity of the analysis. The limitations of direct microscopic observation of individual cells are now being addressed through the development of “emerging technologies”, as a supplement or alternative to conventional techniques. With respect to HAB detection, these “emerging technologies” can be generally divided into “taxon-specific” and “toxin-specific” probes. In this context, a probe may be defined as a molecular tool used to delineate a particular group of organisms (taxon-specific), or a suite of toxic metabolites (toxin-specific) produced by these organisms. In all cases, these probes are configured to react with or hybridise to a molecular “target”, such as a particular toxin or nucleic acid sequence that is characteristic of the organism in question.

Probe design and configuration is a complex and sophisticated process, but once developed, the probe can usually be applied with relatively rudimentary training by non-experts. Thus they are ideal potential candidates for use in routine monitoring programs. Taxon-specific nucleic acid probes that have been developed for a few HAB taxa include methods applicable to whole cells (e.g., *in situ* hybridization) and cell homogenates (e.g., dot blotting, polymerase chain reaction [PCR], restriction fragment length polymorphism [RFLP] and “sandwich” hybridization assays). Further developments are anticipated in the use of DNA microarrays (multichannel “chips”) that can be read diagnostically for expressed gene products in automated chip readers. At the moment, sandwich hybridization assays are particularly amenable to the rapid screening of large numbers of samples of HAB taxa because the reactions are simple to perform,

nucleic acid purification is not required, and the process may be readily automated. This latter attribute is particularly important in effort to develop real-time observation systems.

Available toxin-specific probes for HAB species at the cellular level include antibody methods based upon *in situ* immunolocalization using immuno-gold and transmission electron microscopy (TEM) and fluorescence-labeled antibodies that can be observed under epifluorescence microscopy. Obviously, these techniques are tedious to perform and cannot yet be considered as a routine monitoring tool. Other immuno-technologies that have been developed for screening HAB toxins in cell homogenates include enzyme-linked immunosorbant assays (ELISA) in cuvettes or multi-well plates, enzyme linked immunofiltration assays (ELIFA) performed on a test strips, and the recently developed lateral flow immunochromatographic (LFI) assay (MIST Alert^{TM}). The latter technique has been extensively tested for screening toxins in mixed field plankton assemblages including HAB species. In addition, field trials on the use of radio-receptor assays for certain key HAB toxins (ASP, PSP, brevetoxins) have also shown considerable promise as monitoring tools.

Most taxon- and toxin-specific probes are designed in the laboratory and first applied to samples from cultured organisms. Extrapolation to field samples is not always easy or efficacious due to matrix effects, low target cell concentrations, defining appropriate general reaction conditions and lack of knowledge regarding probe specificity. Furthermore, all probes are initially configured to work on discrete samples with detection in a manual mode. Nevertheless, there have been recent advances in the development of automated systems that can be deployed on moorages in the field ("cytobuoys"). In principle, probes may be configured to yield a signal in a number of detection modes (fluorescence, absorbance, chemiluminesce, radioactive decay, etc.), and thus offer the possibility for automation and even field deployment using the appropriate detector. Such systems are not in general circulation and must be regarded as prototypes, but they offer excellent potential for *in situ* semi-continuous monitoring of target analytes in virtual real time. Combination of such emerging probes with optical, chemical and physical sensors will be a reality within the next few years. Hence, this topic is highly appropriate for an advanced workshop on real time observation systems, with particular reference to HAB detection. Both theory and practice of these emerging technologies will be presented orally and accompanied by the relevant tutorials and live demonstration sessions at the workshop.

2.3 Modelling

Early warning and prediction of algal blooms requires observations to characterise algal distributions in relation to environmental factors (e.g., advection, mixing, light, nutrients), and models that relate algal population dynamics to the observed properties of the environment. Models can range from empirical predictions (e.g., blooms will occur after major runoff events) to detailed numerical forecasts based on simulations of algal growth and behaviour in hydrodynamic models. Predictive models can be developed and validated only if appropriate observations are available. Thus, physical-chemical-biological observation systems, linked in a quantitative way to models, are essential to early warning and prediction of algal blooms.

Important decisions relevant to mitigation of harmful blooms rely on empirical or conceptual models relating algal population dynamics to environmental forcing, such as climate variability (e.g., El Niño) or human influences such as nutrient loading. Simulation models can be effective in revealing which factors dominate in the control of algal bloom dynamics. Water quality models incorporate information on many processes that influence the distributions of nutrients, oxygen, phytoplankton and light. Rarely can the models be parameterised using data from the environment to be modelled, or the target species to be considered. Consequently, although species groups can be treated simultaneously, the conditions that lead to the dominance of a particular species are difficult to resolve with such detailed, but still generalised, models. Nevertheless, multi-parameter simulation models can be an important tool to explore the controls (e.g., nutrient loading, light, tidal flushing) on the biomass and growth rates of phytoplankton in particular environments. The development of optically-based ecosystem models, in which the physically-forced evolution of biological fields is directly simulated on the basis of optical properties, has important implications for the development of real-time observation and prediction systems in coastal waters (Bissett *et al.* 2001). Development of such systems is in its early stages; progress depends not only on the development of optical observation systems and advances in ecological modelling, but also on significant improvements in capabilities for assimilation of data products into models. Clear appreciation of the requirements, capabilities, and limitations of data assimilation techniques is therefore required to develop advanced modelling capabilities in coastal observation systems.

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3 Format of the Workshop

The workshop will be designed to welcome a total of up to 90 attendees, including about 40 invited lecturers and demonstrators. Selection of the 50 participants/contributors will favour students and end users of coastal observation systems; a primary criterion for selection will be the degree of benefit the applicant expects to derive from knowledge gained from the workshop. The workshop will last 10 days and include the following activities:

a) Plenary sessions with overview lectures complemented by contributed oral presentations and debates. The scope of these presentations will extend beyond observation systems to real-time modelling and prediction. The plenary sessions will take place in a conference room which can host up to 180 persons (see Part C).

b) Poster sessions to extend over several days. Computer-based presentations of real-time observations will be encouraged.

c) Practical tutorials with demonstrations in the laboratory and in the field. Each tutorial is presented several times to a different group of about 10 - 12 participants. The tutorials will take place on different platforms (laboratory, pier, small and medium size boats). Demonstrations of real-time data acquisition will be given using different operational systems through internet (MAREL, Seawatch, …).

d) Intercomparisons and practical demonstrations of instruments. Sustained simultaneous deployment of instruments throughout the workshop would be encouraged. As far as is practical, manufacturers would be given the opportunity to demonstrate instruments or systems. Emerging technologies will also be presented through demonstrations.

e) Industrial exhibitors will be allowed to advertise their instruments and observation systems at their own expense.

Examples of lecture topics:

- Phytoplankton optical properties (including fluorescence and absorbance), and applications to HABs
- Seawater apparent optical properties, with emphasis on HABs
- Innovative optical approaches (e.g., flow cytometry, particle counting, high resolution imager, …)
- Other *in situ* sensors useful for monitoring and predicting HABs
- Strategy in the use of *in situ* sensors according to HABs characteristics
- Ocean Colour theoretical background, algorithms, sensors, and applicability for the detection and monitoring of HABs
- Remote sensing of sea surface physical properties, and applications in the context of HABs
- Development and deployment of an instrumented mooring
- Observation networks
- HABs ecosystem modelling
- Data assimilation
- Operational oceanography

Examples of tutorials:

- Calibration and maintenance
- Instrument deployment
- Data acquisition and processing
- Acquisition of remotely sensed data
- Image processing
- Practical aspects and maintenance of a mooring
- Real-time data acquisition, processing and banking from an instrumented mooring
- Demonstration of a HABs ecosystem model
- Demonstration of data assimilation techniques

4 Workshop Programme

In order to cover the various aspects of real-time observation systems in an optimal way, we considered the following guidelines:

- To cover all operational real-time observation techniques used in the coastal environment, through overview
- To provide thorough descriptions, through lectures and tutorials, of the techniques that are near-operational and for which *in situ* sensors have been recently commercialised (the case for several optical sensors).
- To overview the promising emerging approaches and technologies for which no instrument is commercially available (through demonstrations).
- To maintain a good balance between optical, chemical and physical approaches for real-time observation of the HABs environment.

PART 1: *In situ* sensors		
11/6	**08:45-09:15**	**Introduction**
	09:15-10:00	**Lecture No. 1:** Overview on observation and prediction of HABs
	10:00-10:45	**Lecture No. 2:** Overview on physical and chemical dynamics of coastal ecosystems
	10:45-11:15	*Coffee Break*
	11:15-12:00	**Lecture No. 3:** Overview of optical observation of biological variability
	12:00-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-18:30	**POSTER SESSION**[2]
12/6	**08:30-09:15**	**Lecture No. 4:** Theory and state-of-the-art on optical properties of phytoplankton and other marine substances, with emphasis on HABs
	09:15-09:45	**Contributed oral presentation No. 1**[3]
	09:45-10:15	**Discussion**
	10:15-10:45	*Coffee Break*
	10:45-11:30	**Lecture No. 5:** Description of the different methods for *in situ* measurement of inherent optical properties, and assessment of their potential for HABs detection
	11:30-12:00	**Contributed oral presentation No. 2**
	12:00-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-18:30 *coffee break*: **16:00-16:30**	**Tutorial No. 1:** Calibration and maintenance of inherent optical properties sensors (1 hours 4 times, 4 groups rotating)
		Tutorial No. 2: Deployment of inherent optical properties sensors (profiling and continuous underway sampling) (1 hours 4 times, 4 groups rotating))
		Tutorial No. 3: Deployment of inherent optical properties sensors on mooring (1 hours 4 times, 4 groups rotating)
		Tutorial No. 4: Data acquisition and processing of inherent optical properties sensors (1 hours 4 times, 4 groups rotating)

[2] This session will allow the participants to have a first look at the posters and give them the opportunity to meet with the authors. The posters will then remain in place until the end of the workshop.

[3] Contributed oral presentations (and posters) will illustrate and complete the content of lectures.

13/6	**08:30-09:30**	**Lecture No. 6:** Theory and current literature on, and *in situ* measurement of the phytoplankton fluorescence, with emphasis on HABs
	09:30-10:00	**Contributed oral presentation No. 3**
	10:00-10:30	*Coffee break*
	10:30-11:00	**Contributed oral presentation No. 4**
	11:00-11:30	**Contributed oral presentation No. 5**
	11:30-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-18:30 *coffee break*: **16:00-16:30**	**Tutorial No. 5:** Fluorometer (1 hours 4 times, 4 groups rotating)
		Tutorial No. 6: Natural fluorescence (1 hours 4 times, 4 groups rotating)
		Tutorial No. 7: Variable fluorescence (1 hours 4 times, 4 groups rotating)
		Tutorial No. 8: Deployment (1 hours 4 times, 4 groups rotating)
14/6	**08:30-09:15**	**Lecture No. 7:** Measurement of seawater reflectance and vertical attenuation coefficient, with emphasis on HABs
	09:15-09:45	**Contributed oral presentation No. 6**
	09:45-10:15	**Discussion**
	10:15-10:45	*Coffee break*
	10:45-11:30	**Lecture No. 8:** Overview of chemical and physical sensors
	11:30-12:15	**Lecture No. 9:** Assessment of zooplankton
	12:15-12:45	**Discussion**
	12:45-14:00	*Lunch*
	14:00-18:30 *coffee break*: **16:00-16:30**	**Tutorial No. 9:** Calibration, maintenance, and deployment of optical sensors for the measurements of reflectance and the vertical attenuation coefficient (2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 10: Data processing (2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 9': Calibration, maintenance, and deployment of optical sensors for the measurements of reflectance and the vertical attenuation coefficient(2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 10': Data processing (2 hours 2 times, 2 of the 4 groups rotating)

16/6	**08:30-09:15**	**Lecture No. 10:** Emerging technologies for HABs study / monitoring
	09:00-10:00	**Lecture No. 11:** Emerging technologies for HABs study / monitoring
	10:00-10:30	*Coffee break*
	10:30-11:00	**Contributed oral presentation No. 7:** Emerging technologies for HABs study / monitoring
	11:00-11:30	**Contributed oral presentation No. 8:** Emerging technologies for HABs study / monitoring
	11:30-12:00	**Contributed oral presentation No. 9:** Emerging technologies for HABs study / monitoring
	12:00-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-18:30 *coffee break*: **16:00-16:30**	**DEMONSTRATIONS**[4]

[4] While tutorials will be given in a more formal way for techniques that are operational or near-operational and for which instruments are newly available on the market, open demonstration booths will allow the participants to discover various emerging technologies which may become accessible to the users in the near future.

PART 2: Remote Sensing		
17/6	**08:30-09:15**	**Lecture No. 12:** Bio-optical modelling and derived products
	09:15-09:45	**Contributed oral presentation No. 10**
	09:45-10:15	***Coffee break***
	10:15-10:45	**Discussion**
	10:45-11:30	**Lecture No. 13:** Overview of Ocean Colour theoretical background, sensors, and applicability for the detection and monitoring of HABs (capabilities, limitations)
	11:30-12:00	**Contributed oral presentation No. 11**
	12:00-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-18:30 *coffee break*: **16:00-16:30**	**Tutorial No. 11:** Ocean colour data acquisition (from antenna or internet) and image processing (4 hours, group 1 of 4)
		Tutorial No. 11': Ocean colour data acquisition (from antenna or internet) and image processing (4 hours, group 2 of 4)
		Tutorial No. 11'': Ocean colour data acquisition (from antenna or internet) and image processing (4 hours, group 3 of 4)
		Tutorial No. 11''': Ocean colour data acquisition (from antenna or internet) and image processing (4 hours, group 4 of 4)

PART 3: Real Time Observation Systems

18/6	**08:30-09:15**	**Lecture No. 14:** Development and deployment of an instrumented mooring
	09:15-09:45	**Contributed oral presentation No. 12**
	09:45-10:15	**Discussion**
	10:15-10:45	***Coffee break***
	10:45-11:30	**Lecture No. 15:** Underway systems
	11:30-12:15	**Lecture No. 16:** Glider and AUV observation systems
	12:15-12:30	**Discussion**
	12:30-14:00	***Lunch***
	14:00-18:30 *coffee break*: **16:00-16:30**	**Tutorial No. 12:** Design, maintenance and other practical aspects of a mooring (power supply, communication, bio-fouling, …) (2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 13: Real-time data acquisition, processing and banking from an instrumented mooring (2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 12': Design, maintenance and other practical aspects of a mooring (power supply, communication, bio-fouling, …) (2 hours 2 times, 2 of the 4 groups rotating)
		Tutorial No. 13': Real-time data acquisition, processing and banking from an instrumented mooring (2 hours 2 times, 2 of the 4 groups rotating)
19/6	**08:30-09:15**	**Lecture No. 17:** Overview of observation networks
	09:15-10:00	**Lecture No. 18:** Overview on bio-fouling
	10:00-10:30	**Discussion**
	10:30-11:00	*Coffee break*
	11:00-12:30	**Tutorial No. 14-17:** Demonstration of an observation network[5] (1.5 h, 4 separate sessions)
	12:30-14:00	*Lunch*
	14:00-19:00 *coffee break*: **17:00-17:30**	**Tutorial No. 14-17:** Demonstration of an observation network (1.5 h, 4 separate sessions, groups rotating)
		Tutorial No. 14-17: Demonstration of an observation network (1.5 h, 4 separate sessions, groups rotating)
		Tutorial No. 14-17: Demonstration of an observation network (1.5 h, 4 separate sessions, groups rotating)

[5] In Tutorials 14-17, 4 different kinds of observation networks (to be determined) will be presented.

PART 4: Modelling

20/6	**08:30-09:15**	**Lecture No. 19:** Modelling[6]
	09:15-10:00	**Lecture No. 20:** Modelling
	10:00-10:30	*Coffee break*
	10:30-11:15	**Lecture No. 21:** Modelling
	11:15-12:00	**Lecture No. 22:** Modelling
	12:00-12:30	**Discussion**
	12:30-14:00	*Lunch*
	14:00-14:45	**Lecture No. 23:** Theoretical bases of various data assimilation techniques
	14:45-18:30 *coffee break*: **16:00-16:30**	**DEMONSTRATIONS**[7]
21/6	**09:15-10:00**	**Lecture No. 24:** The point of view of users[8]
	10:00-10:30	**Discussion**
	10:30-11:00	*Coffee break*
	11:00-11:45	**Lecture No. 25:** Prospective for observation systems
	11:45-12:15	**Discussion**
	12:15-14:00	*Lunch*
	14:00-16:00	**Synthesis and recommendations**

Special suggestions for contributed lectures:

- Toxin detection
- Taxonomic probes
- Other "biosensors"
- Acoustic and other means of detecting zooplankton
- Oxygen electrodes
- Primary productivity measurements in real time
- Particle size distribution and application to early warning systems

Notes:

- This program involves a total of 25 lecturers, 24 tutors and 12 contributed presentations. If we account for the fact that some tutors will contribute to more than one tutorial and that some lecturers may be involved in tutorials, the sum of lecturers and tutors is expected to be around 40.

[6] In Lectures 19-22, 4 different kinds of models (to be determined) will be presented according to lecturers who will accept our invitation.

[7] Various models will be presented in demonstration booths by animated simulations, examples of real-time utilisation, …

[8] Here, what the users expect and how this workshop was useful with regard to these expectations will be presented.

- Most of the lectures will last 45 minutes and contributed presentations 30 minutes. Contributors will be asked to submit abstracts for poster presentations, and contributed oral presentations will be solicited from selected authors, based on relevance to the program.
- There will be a day-off on Sunday, June 15. Social activities will be organised during this day.
- Exhibitors (mostly instrument manufacturers) will be allowed to present their products during the whole duration of the Workshop at their expense.

5 Facilities

The plenary sessions of the Workshop will take place at the auditorium of the Citadelle de Villefranche-sur-Mer which can host up to 180 persons (see http://www.villefranche-sur-mer.com/htmlgb/4congr_body.html). Villefranche-sur-Mer is nearby Nice which is easily accessible through its international airport. Lodging facilities in Villefranche-sur-Mer are all within 10 minutes walking distance, and there numerous cheap restaurants where the participants will have their meals at choice (see http://www.villefranche-sur-mer.com/htmlgb/fs_accueil.html).

The tutorials, demonstrations and poster sessions will take place at the Observatoire Océanologique de Villefranche (OOV), at 5 minutes walking distance from the Citadelle. All necessary classrooms and laboratories will be available as well as a small pier and small boats for instrument deployment in the Bay of Villefranche.

All facilities are located within 5-10 minutes walking distance.

6 Registrations and Participant Profile

Participants will be selected by the organisation team based on the review of applications (form will be made available on the Workshop web site). In this application, the candidates will be asked to explain how they will use the knowledge gained during the Workshop. Those who will show that they will effectively use their new knowledge in the near-future will be selected in priority.

The main target participants are:

- graduate and post-graduate students who wish to use the technologies and approaches presented during the workshop
- scientists involved in HABs and/or coastal dynamics research who wish to extend the array of tools they use, or wish to better understand and interpret the data they collected using these tools
- scientists involved in HABs and/or coastal dynamics research who actively prospect for the development of a real-time coastal observing system and the use of modelling tools
- scientists, managers or lead technicians involved in or considering operation coastal observation systems

7 Proceedings

An editorial committee will be formed. A new model of publication will be explored and implemented. Recognising innovations in digital communications, rapidly expanding access to the Internet, fast-moving progress in the field of ocean observation and modelling, and the nature of the topic (real-time presentation of large amounts of data, including innovative visualisation techniques), proceedings will be disseminated on a multimedia platform.

Speakers will be asked to use PowerPoint or similar software to prepare digital presentations. Annotated versions of the presentations with links, animations, etc. will be compiled in a CD-ROM and posted on a web site and maintained for a minimum of two years. Illustrated extended abstracts of posters will also be included. Technical guidelines will be provided, and presentations will be edited for consistency. Additionally, a number of forecast simulations will be built and added to the proceedings. These simulations will include real-time connection to operational data servers, download of data, model calculations, and animated illustration of the forecast on maps. This new model will require careful development through consultation. Resources will be required for technical aspects of the digital material.

The proceedings will also be published in a volume of the UNESCO series "Monographs on oceanographic methodology", with M. Babin and C. Roesler as Editors.

8 Organisation team

The organisation of the Workshop will be managed by a convenor: Marcel BABIN (the co-ordinator of the current proposal), assisted by a co-convenor: John Cullen from the Department of Oceanography at Dalhousie University (Halifax, Canada). An organisation committee (OC) will assist the convenors in defining the scientific themes, and the format and programme (including the list of lecturers and tutors) of the Workshop. This committee is composed of the following scientists:

Name	Affiliation	Expertise
AIKEN, Jim	Plymouth Marine Laboratory, Plymouth, UK	Marine optics, remote sensing
CEMBELLA, Allan	Institute for Marine Sciences, National Research Council, Halifax, Canada	HAB ecophysiology
CLAUSTRE, Hervé	Lab. d'Océanographie de Villefranche, Univ. Pierre et Marie Curie / CNRS, Villefranche, F	Phytoplankton ecology and marine optics
DICKEY, Tommy	Univ. Of California in Santa Barbara, USA	Marine optics, development of instrumented mooring in oceanography
LEE, Joseph Hun-wei	University of Hong Kong, China	HAB monitoring and management
ROESLER, Collin	Bigelow Laboratory for Ocean Sciences, USA	Marine optics
KARLSON, Bengt	Swedish Meteorological and Hydrological Institute Oceanographic Services, Västra Frölunda, SE	HAB monitoring and management
FOURNIER-SICRE, Vincent	ACRI-ST, F	Marine optics *in situ* measurements and data processing, earth observation data processing

In this committee, there are representatives of both the experts (marine optics, real-time observation) and users communities. The Chair of the Scientific Steering Committee of the GEOHAB program, Patrick GENTIEN from the Centre de Recherche en Ecologie Marine et Aquaculture (IFREMER/CNRS, l'Houmeau, France), will contribute to the work of the OC.

The organisation team will be assisted by a sub-contractor specialised in the organisation of international meeting. The sub-contractor will be in charge of:

- creating the Workshop web site
- managing the registrations (through the web site)
- advertising the Workshop
- doing arrangements for hotel reservations in Villefranche-sur-Mer
- distributing the Proceedings
- any other practical logistics, including individual Workshop schedules for the participation to tutorials